

WEB SUPPLEMENT

w1. Research question

A well-defined research question tailors the systematic review. Before formulating the research question, find out whether others are working on the same topic by checking whether the same or a similar topic has been registered at PROSPERO, the international database of prospectively registered systematic reviews, and make sure you are up to date with recent systematic reviews on the topic. If others have not addressed your topic recently you are ready to proceed and break down your research questions into the four most important concepts defined by PICO. If there are recent reviews on a similar topic, you can refine your research question to differentiate it from existing reviews.

Example: We want to study the associations between route of administration, dose, timing of initiation and duration of menopausal hormone therapy, and cardiovascular risk among postmenopausal women. We have identified a previously published, more specific systematic review investigating the effects of nonoral postmenopausal hormone therapy on markers of cardiovascular risk ¹. Because you are interested in administration route, dose, timing, and duration of hormone therapy your research question is broader and you should continue with the project. Now, break down the research question using the PICO model ². Keep in mind that not every research question can be translated into the PICO mode most suitable for therapeutic research questions. Including every PICO item in your search query may risk the loss of relevant publications. Menopausal hormone therapy is only given to women in menopause. Thus in this example the search need not include the terms postmenopausal and women. The search should not include overly specific terms that are potentially not mentioned in the title and abstract—in this example route of administration, dose, timing of initiation, and duration of the therapy. These topics will most likely be discussed in the full text of any article on menopausal hormone therapy.

Research question: What is the association between administration route, dose, timing of initiation and duration of menopausal hormone therapy, and cardiovascular risk among postmenopausal women?

P	Patient problem/Population	Postmenopausal women
I	Intervention	Menopausal hormone therapy (administration route, dose, timing of initiation, and duration)
C	Comparison (if there is one)	No treatment
O	Outcome(s)	Atherosclerosis, peripheral arterial disease, carotid intima-media thickness, stroke, transient ischemic attack, heart failure, coronary heart disease, angina, chest pain, venous thromboembolism

w2. Developing a search strategy

Developing the search strategy starts with analysis of the research question. Which of the elements in the research question are most important in the search? The search should consist of as few elements as possible to optimize the sensitivity of the search results. Two elements should be taken from the research question in the example above: menopausal hormone therapy and cardiovascular risk.

Each element chosen is then translated into keywords used by the databases. Two major sets of keywords exist: PubMed MEDLINE uses MeSH (medical subject headings) terms while articles in Embase are assigned Emtree terms. These thesauruses are the starting point for collecting potentially relevant search terms to formulate the search strategy. From the thesauruses, not only should terms themselves be collected but also their synonyms (or entry terms in MEDLINE). Using truncation (most databases use the asterisk * for that purpose); variations of words can be searched simultaneously.

The collected terms are then combined into a search strategy using the Boolean operators OR between synonyms and AND between elements. Some databases offer more complex operators such as proximity operators, which allow the searcher to search sets of terms within a specified distance of each other. This can greatly increase the retrieval of relevant references. Parentheses determine priority in the searches. Generally, OR should be executed before AND and each element therefore starts and ends with parentheses. The search should use field codes appropriate for the databases, expand thesaurus terms (including the narrower terms in the tree), and limit searching of non-thesaurus terms to title and/or abstract, and author keywords.

If you have no experience with literature searches, the best way to learn is to start with PubMed. It has the simplest search engine, and once you learn how to search PubMed it will be easier to search other databases. A search for systematic reviews should, however, never be confined to a single database. We also recommend that searches for all systematic reviews are best performed by experienced medical information specialists.

Detailed instructions on how to properly perform systematic literature search can be found in a recently published methodological paper ³. Also, there are numerous online tutorials you may consult to learn basic principles of searching the literature. We suggest the PubMed online training, which is free, and can be found at <https://learn.nlm.nih.gov/documentation/training-packets/T0042010P/>. A very good online tutorial that follows a very systematic method can be found at libguides.vu.nl/PMroadmap.

w3. Example of search strategy

Here is an example of a search strategy for a recently published meta-analysis of intervention studies that evaluated the association between phytoestrogen supplementation followed by regular, normocaloric diet and glucose homeostasis, and type 2 diabetes risk in adult women. A literature search was done using five electronic databases: Medline via Ovid, EMBASE, Web of Science Core Collection, Cochrane CENTRAL via Wiley, and Google Scholar. This example reflects the complexity of search strategy. You can compare the search syntaxes among different medical databases ⁴.

Embase.com	('phytoestrogen'/exp OR 'soybean'/de OR 'soybean extract'/de OR 'soybean protein'/de OR 'soybean oil'/de OR 'soybean meal'/de OR 'soybean milk'/de OR 'red clover'/de OR 'red clover extract'/de OR 'isoflavone'/de OR 'isoflavone derivative'/de OR 'daidzein'/de OR 'genistein'/de OR 'soy food'/de OR (phytoestrogen* OR phytoestrogen* OR phyto-estrogen* OR phyto-oestrogen* OR soybean* OR soy OR 'red clover' OR Trifolium OR Isoflavon* OR daidzein OR genistein* OR flaxseed OR ((herb* OR plant*) NEAR/6 (estrogen* OR estrogen*)) OR tofu):ab,ti) AND ('diabetes mellitus'/exp OR 'insulin response'/exp OR 'glucose blood level'/exp OR 'insulin blood level'/exp OR hyperinsulinism/exp OR (diabet* OR ((glucose OR sugar OR insulin*) NEAR/6 (level* OR blood OR serum OR plasma* OR concentration*)) OR glucosaem* OR glucosem* OR glycaem* OR glycem* OR hyperinsulin* OR hypoinsulin* OR insulinaem* OR insulinem* OR (insulin NEAR/3 (response OR dependen* OR resistan* OR sensitiv*))):ab,ti) NOT ([animals]/lim NOT [humans]/lim) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim)
Medline Ovid	(exp "Phytoestrogens"/ OR "Soybeans"/ OR exp "Soybean Proteins"/ OR exp "Soy Foods"/ OR "Trifolium"/ OR exp "Isoflavones"/ OR (phytoestrogen* OR phytoestrogen* OR phyto-estrogen* OR phyto-oestrogen* OR soybean* OR soy OR "red clover" OR Trifolium OR Isoflavon* OR daidzein OR genistein* OR flaxseed OR ((herb* OR plant*) ADJ6 (estrogen* OR estrogen*)) OR tofu).ab,ti.) AND (exp "Diabetes Mellitus"/ OR "Insulin Resistance"/ OR glucose/bl OR insulin/bl OR exp Hyperinsulinism/ OR (diabet* OR ((glucose OR sugar OR insulin*) ADJ6 (level* OR blood OR serum OR plasma* OR concentration*)) OR glucosaem* OR glucosem* OR glycaem* OR glycem* OR hyperinsulin* OR hypoinsulin* OR insulinaem* OR insulinem* OR (insulin ADJ3 (response OR dependen* OR resistan* OR sensitiv*))).ab,ti.) NOT (exp animals/ NOT humans/) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt.
Cochrane	((phytoestrogen* OR phytoestrogen* OR phyto-estrogen* OR phyto-oestrogen* OR soybean* OR soy OR 'red clover' OR Trifolium OR Isoflavon* OR daidzein OR genistein* OR flaxseed OR ((herb* OR plant*) NEAR/6 (estrogen* OR estrogen*)) OR tofu):ab,ti) AND ((diabet* OR ((glucose OR sugar OR insulin*) NEAR/6 (level* OR blood OR serum OR plasma* OR concentration*)) OR glucosaem* OR glucosem* OR glycaem* OR glycem* OR hyperinsulin* OR hypoinsulin* OR insulinaem* OR insulinem* OR (insulin NEAR/3 (response OR dependen* OR resistan* OR sensitiv*))):ab,ti)
Web of science	TS=(((phytoestrogen* OR phytoestrogen* OR phyto-estrogen* OR phyto-oestrogen* OR soybean* OR soy OR "red clover" OR Trifolium OR Isoflavon* OR daidzein OR genistein* OR flaxseed OR ((herb* OR plant*) NEAR/5 (estrogen* OR estrogen*)) OR tofu) AND ((diabet* OR ((glucose OR sugar OR insulin*) NEAR/5 (level* OR blood OR serum OR plasma* OR concentration*)) OR glucosaem* OR glucosem* OR glycaem* OR glycem* OR hyperinsulin* OR hypoinsulin* OR insulinaem* OR insulinem* OR (insulin NEAR/2 (response OR dependen* OR resistan* OR sensitiv*)))) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine) NOT (human* OR patient*))) AND DT=(article)
Google scholar	phytoestrogens soybean soy "red clover" Trifolium Isoflavones diabetes insulin glucose

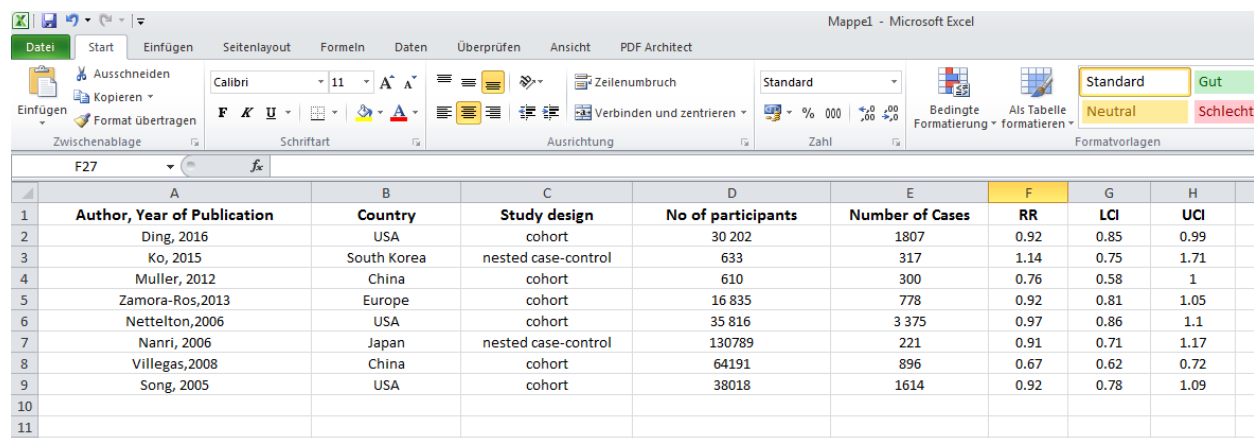
w4. Review checklist including in- and exclusion criteria

In the table below you can find an example checklist for a systematic review investigating the effect of phytoestrogen supplementation on endothelial function and cardiovascular disease risk biomarkers among postmenopausal women that is limited to randomized controlled trials. This review is registered at PROSPERO where you can also find a study protocol (ID: CRD42019121110)

Phytoestrogen supplementation, Endothelial Function and Cardiovascular Disease Risk Biomarkers among Postmenopausal Women: a Meta-analysis of Randomized Controlled Trials
Study Design: Randomized clinical trials
Exposures: Phytoestrogens: red clover, trifolium, isoflavone, daidzein, genistein, soya, all kind of soybean meals; lignans <ul style="list-style-type: none"> ○ Treatment intervention: supplementation, dietary intervention, capsules/tablets and all kind of phytoestrogen interventions
<ul style="list-style-type: none"> ● Outcomes: Cardiovascular Disease Risk Markers: <ul style="list-style-type: none"> ○ Inflammatory markers: TNF-α, homocysteine, leptin, interleukins (1,6 and 8), leukotrienes, histamine, prostaglandins, cytokines, high sensitivity CRP, malondialdehyde (MDA), complement system, angiotensin converting enzyme (ACE); Coagulation system, Fibrinolysis (Plasmin, fibrinogen, thrombin, von Willebrand factor, thrombomodulin) ○ Oxidative stress: reactive oxygen species (ROS), lipid peroxidation, isoprostanes (IsoPs) and malondialdehyde (MDA), 5-lipoxygenase, 12-lipoxygenase, myeloperoxidase ... ○ Serum lipids: cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), non- high-density lipoprotein cholesterol (non-HDL-C), and apolipoprotein A1, apolipoprotein B (Apo-B) ○ Endothelial dysfunction: <i>endothelium-derived relaxing factors (EDRF), nitric oxide (NO), prostaglandin I2 (PGI2), endothelium-derived hyperpolarizing factor (EDHF), endothelium-derived contracting factors (EDCF), endothelin-1 (ET-1),</i> nitrites, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectine, P-selectine ● Atherosclerosis and vascular function: <ul style="list-style-type: none"> ○ Arterial stiffness: pulse wave velocity aortic pulse-wave velocity(PWV), brahial flow-mediated dilation (FMD), peripheral arterial tonometry (PAT) ○ Carotid intima-media thickness, carotid atherosclerotic plaques, carotid atherosclerosis, coronary atherosclerosis
Inclusion criteria: <ol style="list-style-type: none"> 1. Studies conducted among postmenopausal women 2. Studies reporting associations of phytoestrogens with the outcomes outlined above. 3. No language or date restriction.
Exclusion criteria: <ol style="list-style-type: none"> 1. Studies in animals 2. Studies conducted in men 3. RCTs investigating combination of interventions (e.g. phytoestrogens +hypocaloric diet, phytoestrogens+ exercise) 4. Articles with incomplete information. 5. Case-control studies, cohort and cross-sectional studies 6. Systematic review and meta-analysis 7. Meeting/congress reports

w5. Data collection form

The data collection form should contain the most important information you need when analyzing your data. In the example below, you can find a form created in Excel. Included studies are prospective observational studies that reported relative risks (RR), lower (LCI) and upper (UCI) 95% confidence intervals. When pooling the data, you will need number of participants and number of cases. Also, among information you may need it is important to include the name of the lead author and year of publication. What you need will depend on what you plan to do with your data. If you are planning to stratify your analyses you may also extract information on type of study (cohort vs case-control), location, mean age, mean number of participants, and sex.



	A	B	C	D	E	F	G	H
1	Author, Year of Publication	Country	Study design	No of participants	Number of Cases	RR	LCI	UCI
2	Ding, 2016	USA	cohort	30 202	1807	0.92	0.85	0.99
3	Ko, 2015	South Korea	nested case-control	633	317	1.14	0.75	1.71
4	Muller, 2012	China	cohort	610	300	0.76	0.58	1
5	Zamora-Ros,2013	Europe	cohort	16 835	778	0.92	0.81	1.05
6	Nettelton,2006	USA	cohort	35 816	3 375	0.97	0.86	1.1
7	Nanri, 2006	Japan	nested case-control	130789	221	0.91	0.71	1.17
8	Villegas,2008	China	cohort	64191	896	0.67	0.62	0.72
9	Song, 2005	USA	cohort	38018	1614	0.92	0.78	1.09
10								
11								

w6. Review Protocol

The following example illustrates a review protocol, the aim of which was to study whether endogenous sex hormones (ESH) are associated with risk of type 2 diabetes (T2D) in women⁵. The protocol should clearly define each step of the systematic review procedure including the aim of review, inclusion and exclusion criteria, search strategy, data management, statistical analyses, study quality assessment, time frame, and the team members.

PROTOCOL FOR SYSTEMATIC REVIEW

Title: Endogenous Sex-Hormones And Type 2 Diabetes In Women

Authors: Taulant Muka, Jana Nano, Loes Jaspers, Cindy Meun, Wichor Bramer, Albert Hofman, Abbas Dehghan, Maryam Kavousi, Joop Laven, Oscar H. Franco

Leading Author/s: Taulant Muka and Oscar H. Franco

Funding/Support: This study will be sponsored and funded by Metagenics Inc.

Date: January 2015

OBJECTIVES OF THE REVIEW

To summarize the best evidence on (and quantify) the association between endogenous sex hormones and the risk of type 2 diabetes in women.

METHODS

Inclusion and Exclusion Criteria

Inclusion Criteria

1. We will include prospective cohort studies, case-cohort studies, nested case control studies.
2. We will include studies that evaluate total estradiol, bioavailable estradiol, total testosterone, bioavailable testosterone, sex-hormone binding globulin, free androgen index (ratio total testosterone/sex hormone-binding globulin).
3. We will include studies that evaluate the association of sex-hormones (total estradiol, bioavailable estradiol, total testosterone, bioavailable testosterone, sex-hormone binding globulin and free androgen index) with type 2 diabetes mellitus.
4. We will include studies that have been conducted in women.
5. We will include studies conducted in humans.
6. We will include studies conducted in subjects 18+ years old
7. We will not apply language or date restrictions.

Exclusion Criteria

1. We will exclude abstracts, cost effectiveness studies, letters to the editor, conference proceedings, cross-sectional studies, cross-sectional case control studies, systematic reviews, and meta-analyses.
2. We will exclude studies conducted in animals.

Electronic Search Strategy

Electronic databases (Medline, Embase.com, Web of Science, the Cochrane Library, PubMed and Google Scholar) will be targeted. No language restriction or date restriction will be placed on this search. Study identification will include both electronic searching strategies and manual. Electronic searches will involve the electronic databases and search terms listed below. At the same time, we will screen the citations of the relevant studies and will be seeking information from experts in the field.

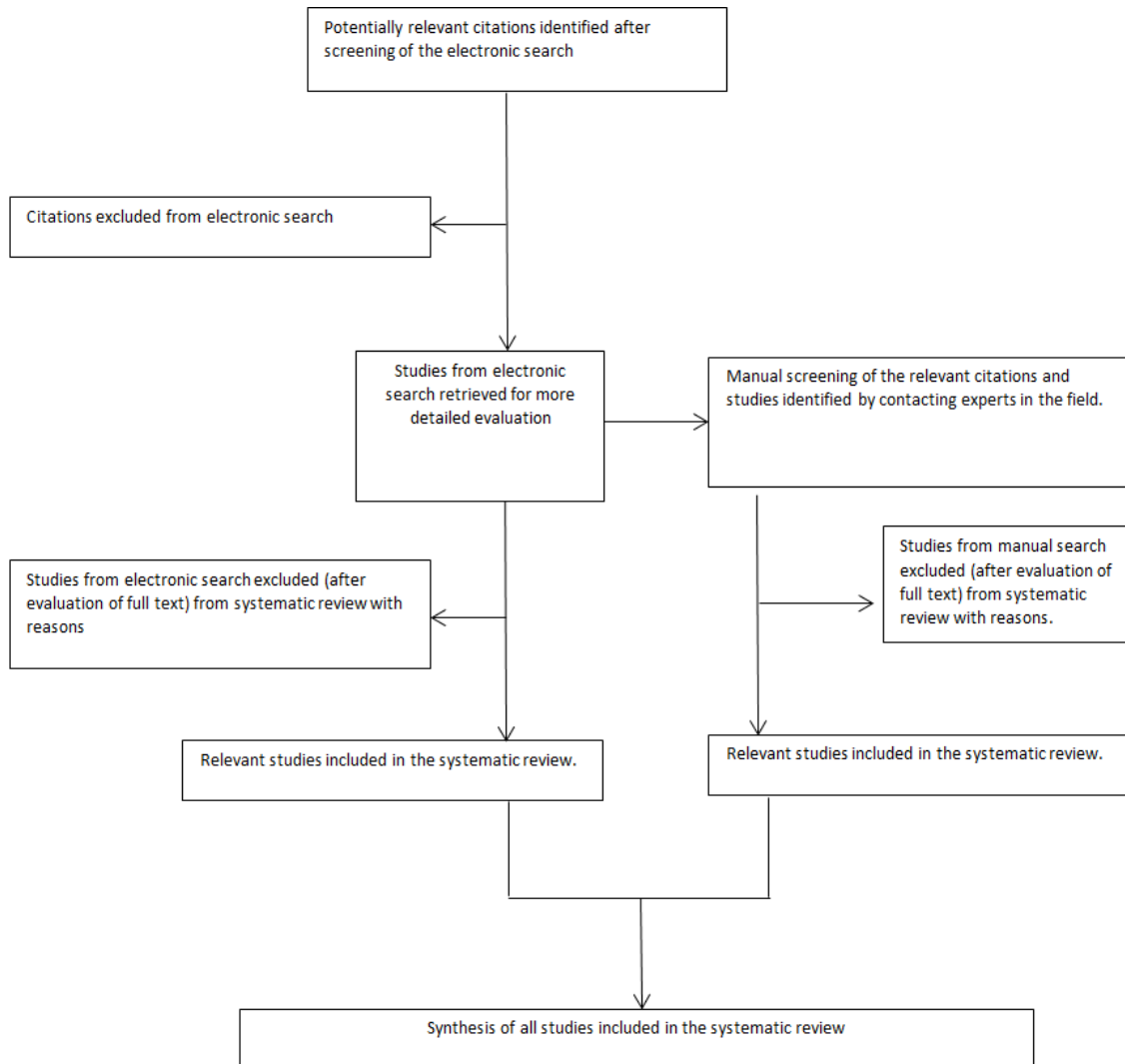
Search Terms

Search terms related to exposure: sex hormone, androgen, testosterone, estradiol, oestradiol, sex-hormone binding globulin, free androgen index.

Search terms related to outcome: type 2 diabetes, non-insulin dependent diabetes mellitus.

Selection Procedure

Studies will be selected for retrieval following the procedure illustrated in **Figure** below.



Data Management

Two independent reviewers working in pairs will screen the titles and abstracts of all studies initially identified according to the selection criteria. Full texts will be retrieved from studies that satisfy all selection criteria. Two authors will independently extract the data and reach consensus, potentially with involvement of a third reviewer. The data extraction form will include information on author, year of publication, study design, baseline population, location, age at baseline, duration of follow-up,

menopause status, exposure assessed, number of subjects included in the analysis, number of cases, summary estimates, adjustments for confounding factors, and sensitivity analysis when appropriate.

Assessment Of Study Quality

Two reviewers will independently rate the quality of studies based on the Newcastle-Ottawa Scale (Stang A et al. 2010). This scale uses a star system with maximum of nine stars to evaluate a study in three domains: selection of participants, comparability of study groups, and ascertainment of outcomes of interest. Studies that receive a score of nine stars will be judged to be at low risk of bias, studies with seven or eight stars will be considered to be at medium risk, and those scoring six or less will be considered to be at high risk of bias.

Data Synthesis And Analysis

For the data we will meta-analyze, the risk estimates of the most adjusted models reported by each study included in the review will be used. To enable a consistent approach to the meta-analysis and enhance interpretation of the findings, relative risks will be converted when appropriate following a previously described method (Chene G et al. 1996). The inverse variance weighted method will be used to combine summary measures using random-effects models to minimize the effect of between-study heterogeneity (DerSimonian R et al. 1986). We will also report the estimates using fixed effect models. Heterogeneity will be assessed using the Cochrane χ^2 statistic and the I^2 statistic (Higgins JP et al. 2003). Publication bias will be evaluated through funnel plots and Egger's regression symmetry tests (Egger M et al. 1997). Study-level characteristics including geographical location, number of total participants, menopause status and risk of bias are prespecified as characteristics for assessment of heterogeneity, and if applicable (depending on the number of studies for each analyses), will be evaluated using stratified analyses and random effects meta-regression (Thompson SG et al. 1999). A narrative synthesis and construction of descriptive summary tables will be done for studies that cannot be quantitatively pooled. All tests will be two-tailed and p-values of 0.05 or lower will be considered significant.

TIME FRAME

The review is expected to take the maximum 6 months.

CONFLICT OF INTERESTS

Reviewers are unaware of any potential conflict of interests

w7. Review registration

PROSPERO is an international database of prospectively registered systematic reviews with health-related outcomes. PROSPERO provides a comprehensive listing of systematic reviews registered at inception to help avoid duplication and reduce reporting bias by enabling comparison of the completed review with what was planned in the protocol. Registration in PROSPERO involves the submission and publication of key information about the design and conduct of a systematic review. The process is computerized and detailed instructions for how to register your review can be found at <https://www.crd.york.ac.uk/prospero/>

Do not register the systematic review before you finalize the study protocol. Reviews are currently accepted for registration as long as they have not progressed beyond the completion of data extraction. Ideally, you should register your review before screening of studies for inclusion begins.

Example: At PROSPERO you can find our recent systematic review protocol under identification number CRD42019121110. You can search PROSPERO in order to find more examples.

w8. Collect all references and abstracts in a single EndNote file

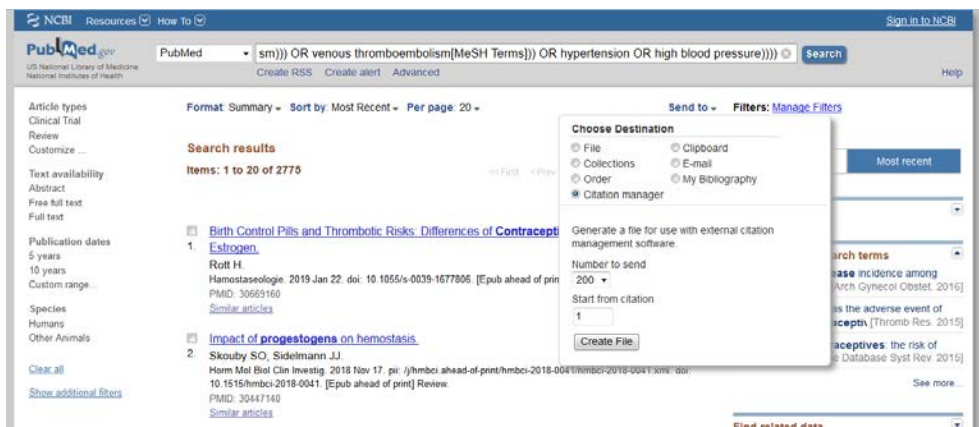
After you run your search strategy, you can export references from any online database to an EndNote file. This process is simple and will greatly simplify your project. Here we give an example of how to export references from the PubMed database (the most commonly used database) to an Endnote file. A comprehensive guide to the reference management software EndNote can be found at <http://aut.ac.nz.libguides.com/endnote/>

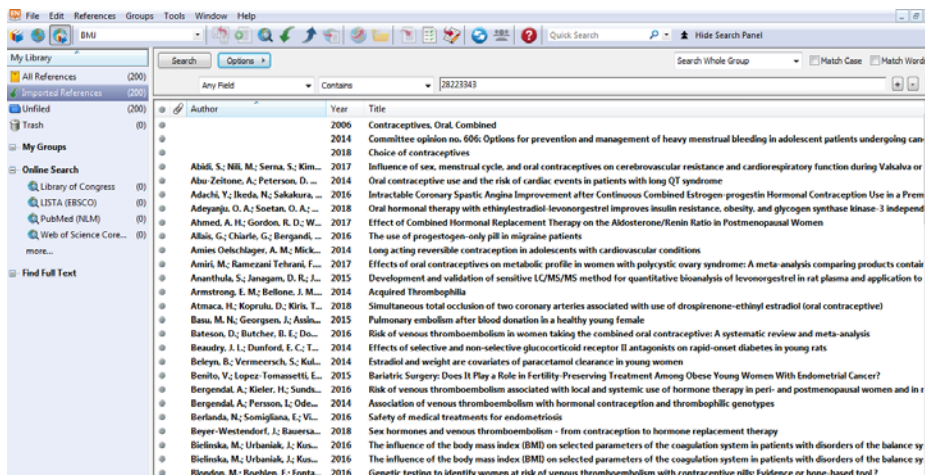
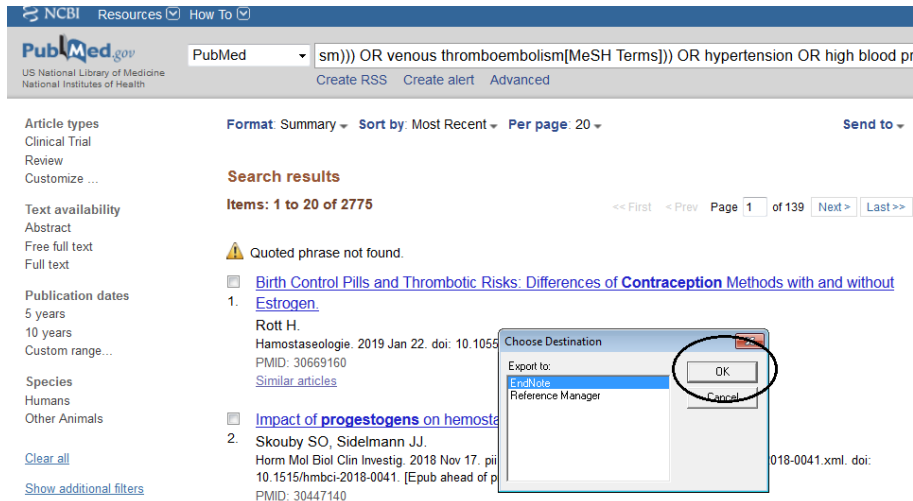
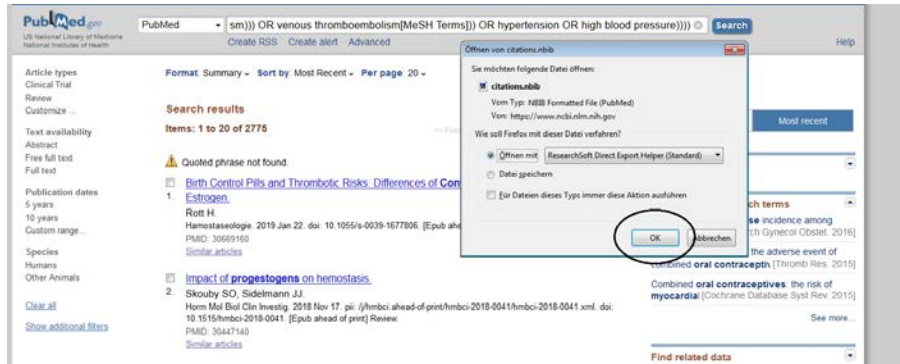
To export references from any PubMed database into EndNote, follow this process:

- A. Make sure your EndNote library is open
- B. Search PubMed database to retrieve relevant results
Copy/paste search strategy in search filed

(((((contraceptives, oral OR ((contraception OR contracept*) AND pill)) AND (progesterin* OR progestational, hormones, synthetic OR progestogen* OR progesterone OR gestagen OR "progesterin only" OR "progestogen only" OR "progestrogen only pill" OR minipill))) AND (((((cardiovascular disease[MeSH Terms] OR heart disease OR stroke OR cerebrovascular disease OR cardiovascular disease OR coronary artery disease OR heart failure OR cardiovascular mortality OR coronary death OR CHD OR CVD OR cardiac death OR myocardial infarction OR angina))) OR ((diabetes OR diabetes mellitus OR DM OR T2DM OR T1DM))) OR (((("venous thromboembolism" OR VTE OR thromboembolism))) OR venous thromboembolism[MeSH Terms])) OR hypertension OR high blood pressure)))

- C. Export references
 - Tick the boxes next to the article titles you wish to export
 - From the top menu, choose **Send to**
 - Check **Citation manager**
 - Click **Create File**
 - **Accept** all next steps and you are done

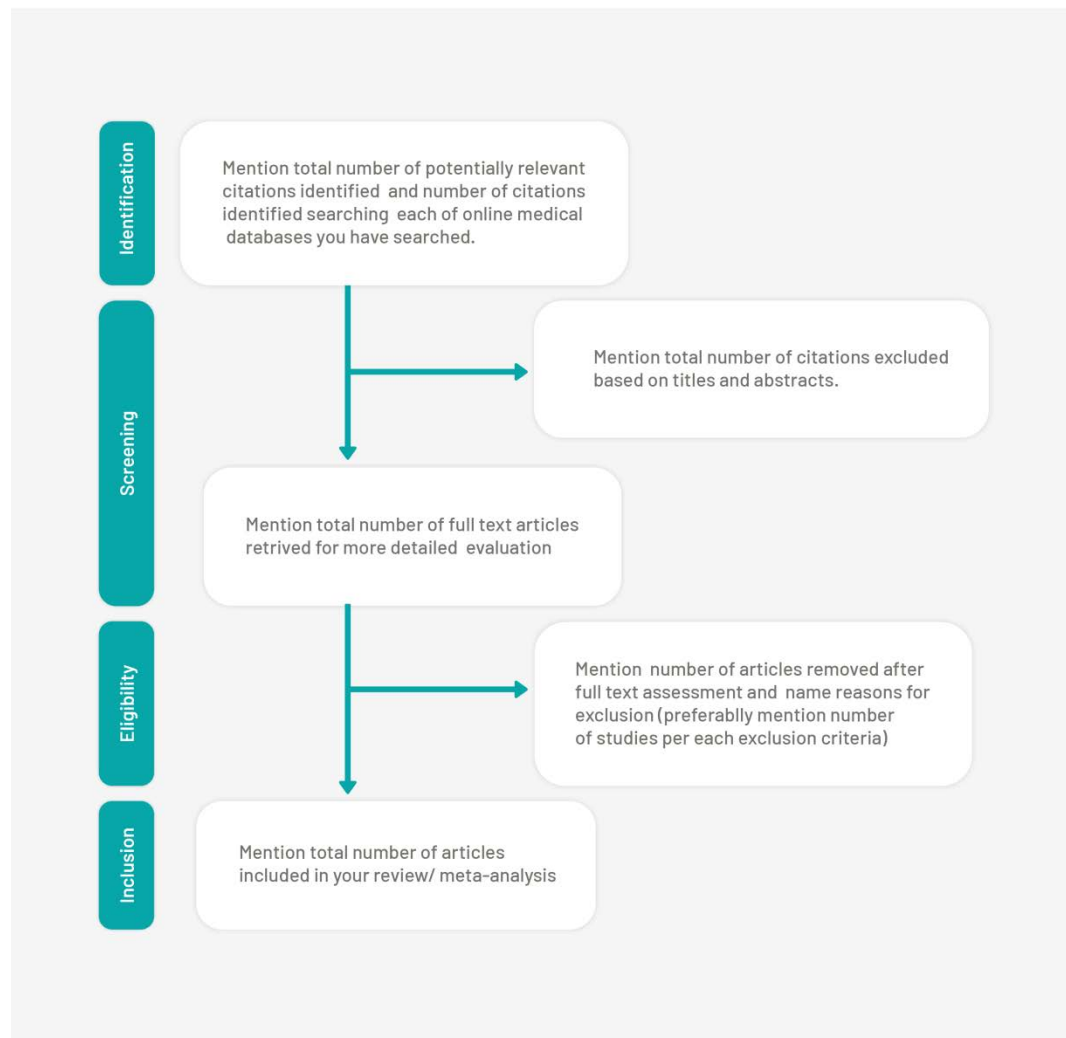




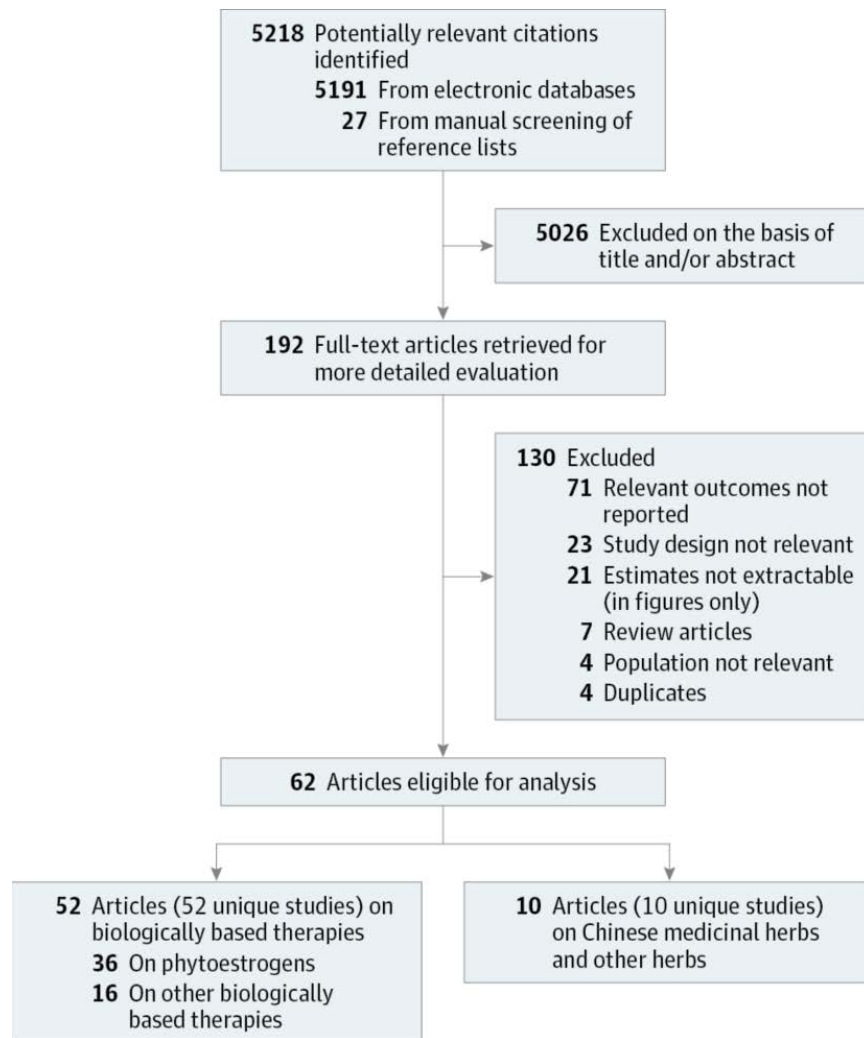
w9. Flow chart design

The flowchart design may vary depending on journal style; however, each flowchart must contain the information provided in the theoretical example below. We also presented a real life example that shows the literature search for identification of randomized clinical trials on the association between use of plant-based therapies and menopausal symptoms ⁶. A full-text assessment usually leads to exclusion of a few hundred studies (in our example, 130 studies were excluded). Because over time it is impossible to remember the reasons for excluding studies, do not forget to note the reasons for exclusion during full-text assessment.

Example 1. Theoretical example



Example 2. Real life example



w10. Example of how to report the risk of bias in RCTs

Author name, year	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of assessment	Missing data	Selective reporting	Other bias
Aubertin-Leheudre et al, 2008 (31)				//////////			//////////
Bakhtiary et al, 2011 (43)							//////////
Blakesmith et al, 2003 (27)						//////////	
Charles, 2009 (40)							
Choquette et al, 2011 (30)		//////////		//////////			//////////
Colacurci et al, 2005 (41)							
Crisafulli et al, 2005 (29)	//////////	//////////		//////////			//////////
Garrido et al, 2006 (37)		//////////					//////////
Hall et al, 2006 (33)		//////////					//////////
Ho et al, 2007 (35)		//////////					//////////
Hutchins et al, 2013 (42)		//////////					
Khaodhiar et al, 2008 (38)							//////////
Kim et al, 2013 (44)							
Liu et al, 2010 (34)							//////////
Liu et al, 2014 (45)							
Nikander et al, 2004 (32)				//////////			//////////
Simao et al, 2014 (28)	//////////			//////////			//////////
Villa et al, 2009 (39)		//////////		//////////			//////////
<p>*Other bias refers to bias due to problems not covered elsewhere in the table (e.g. the study had a potential source of bias related to the specific study design used, or there is insufficient information to assess whether an important risk of bias exists, or insufficient rationale or evidence that an identified problem will introduce bias). White: low risk of bias; pattern //: unclear risk of bias; black: high risk of bias.</p>							

W11. Type of studies, effect measures and model suggestions

Type of Study	Effect measure	Fixed-effect model	Random-effect model
Case-control studies, and cross-sectional and cohort study (with modified assumptions)	Odds ratio (OR) represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure 20842279	Mantel-Haenszel Inverse variance, Peto	Mantel-Haenszel Inverse variance
Randomized controlled trials/clinical trials, cohort studies	Risk ratio (RR)/Rate ratio the "ratio of risk of the outcome in the two groups" 25746068	Mantel-Haenszel	Mantel-Haenszel
Randomized controlled trials/clinical trials, experimental studies	Risk difference (RD)	Inverse variance	Inverse variance
Randomized controlled trials/clinical trials, experimental studies	Mean difference (MD), Standardized mean difference (SMD)	Inverse variance	Inverse variance

w12. Describe included studies

Reviewers may extract data from studies using Word or Excel files and with this information the table of study characteristics can be created, which is customarily needed when performing systematic reviews.

Example: This example is based on a recent meta-analysis of randomized controlled trials investigating the association between use of plant-based therapies and menopausal symptoms ⁶.

Lead Author, Publication Date	Name of study or source of participants	Location	Year of study	Age group	Intervention form, treatment and daily dosage	Intervention period, weeks	Control	Total trial participants	Menopause status
Chinese medicinal herbs									
Fu, 2015 ⁴⁷	Second affiliated hospital of Tianjin University	China	2011	40-60	Chinese herbal medicines	12	Placebo	389	P and PM
Haines, 2008 ⁴⁸	Menopause clinic	Hong Kong	NS	52.0*	Capsule, Dang Gui Buxue Tang, 3 g	24	Placebo	100	PM
Kwee, 2007 ⁴⁹	Local newspapers	Netherlands	2002-2004	53.0*	Liquid extract, Chinese herbal medicines, 90 drops	12	Placebo	31	P and PM
Nedeljkovic, 2014 ⁵⁰	University of Bern	Switzerland	NS	53*	Capsule, Chinese herbal medicines, 3 g	12	Placebo	20	PM
Sluijs, 2009 ⁵¹	Sydney Metropolitan area	Australia	2004-2005	45-65	Tablets, Chinese herbal medicines plus <i>Cimicifuga racemosa</i>	16	Placebo	92	PM
Xia, 2012 ⁵²	Media and referrals	China	2009	45-55	Granule, Chinese herbal medicines, 3.5 g	8	Placebo	72	P
Other medicinal herbs									
Garcia, 2010 ⁵³	Gynaecological clinic	Singapore and Philipines	NS	45-60	Capsule, Nutrafem containing 75 mg of <i>E. ulmoides</i> plant extract and 150 mg of <i>V. radiata</i> plant extract (4 capsules daily)	13	Placebo	159	PM
Heger, 2006 ⁵⁴	Gynaecological clinic	Ukraine	2003-2004	45-55	Tablets, <i>Rheum rhaponticum</i> (ERr 731), 4mg	12	Placebo	110	P

M, menopause; P, peri-menopause; PM, post-menopause;

w13. Data harmonisation

Before performing a meta-analysis and pooling estimates from different studies, we must make sure that we are not mixing apples and oranges. After you complete the data extraction you must compare the effect estimates within included studies. Then you should think carefully about which estimates may be pooled with each other without any manipulation and which of the estimates need some adjustments before pooling with the rest. It is not possible to discuss every case scenario you may face in the future; however, you may find help in most situations online. Here we present a common problem you may encounter when pooling the data from randomized controlled trials (RCTs), which are very often subjected to meta-analysis.

To pool continuous data when performing a meta-analysis, mean values and variance (or standard deviation) are needed. Published trials sometimes report the median, the range of the estimate, and the size of sample. Some authors believe the median may be substituted for the mean, and the Range/4 (for normal distribution) and Range/6 (for any distribution) for the standard deviation. However, neither median nor range formula substitution are appropriate. Hozo and colleagues have developed a method for exactly estimating the mean and the variance when there is no indication of the underlying distribution of the data⁷.

They report that in cases of a sample size >25 it may be possible to use median, yet for small sample size they found that this formula may be used to estimate the mean using the values of the median (m), and low and high end of the range (a and b , respectively).

$$\bar{x} = \frac{a + 2m + b}{4}$$

Using simulated data, they reported that for very small samples (up to 15) the best estimator for the variance is the following formula:

$$s^2 = \frac{1}{12} \left(\frac{(a - 2m + b)^2}{4} + (b - a)^2 \right)$$

When the sample size increases, Range/4 is the best estimator for the standard deviation (and variance) until the sample sizes reach about 70. For large samples (size more than 70) Range/6 is actually the best estimator for the standard deviation (and variance). The formulas can be found in the original article.⁷

The good news is that there is an online calculator that uses these principles to calculate the mean and SD for you. All you need is information on sample size, median of the sample, low and high end of the range of the sample. You can find the calculator online at http://vassarstats.net/median_range.html

Example: We want to investigate the associations between isoflavones and blood C-reactive protein (CRP) levels. For this purpose we will include randomized controlled trials that compare the intervention (isoflavones) and control groups (placebo). To pool the data we need information on mean baseline CRP levels and SD in control and intervention groups in order to calculate the mean difference between start and end of the trial, and to compare if there any difference between intervention and control groups.

The study done by Bakhtiary et al.⁸ is clear because they report mean CRP levels and SDs in each group. However, in a study done by Simao et al.⁹, they report median CRP values and their 25th and 75th percentiles. An inexperienced researcher may exclude this study for lack of a way to pool these two estimates. In **Table 1** you can see the estimates as reported in the original paper by Simao et al, and in **Table 2** you can see the estimates after conversion using the Hozo formula. Another interesting issue you may encounter when preparing data for analysis is differences in metrics. Bakhtiary et al, report CRP in $\mu\text{g/mL}$ and Simao et al, report CRP in mg/L , therefore you must convert the values to the same units before pooling estimates.

Table 1	Baseline Median (25th and 75th percentiles)	End of trial Median (25th and 75th percentiles)
Control Group	4.12 (1.43 – 7.95)	3.75 (0.98 – 5.60)
Intervention Group	8.40 (1.83 – 11.30)	6.55 (1.63 – 10.20)

Table 2	Intervention	Outcome	Intervention Group			Control Group		
			No. of Participants	Baseline mean \pmSD	End study mean \pm SD	No. of Participants	Baseline mean \pm SD	End study mean \pm SD
Bakhtiary et al, 2011	Soy nut 35g/d	CRP ($\mu\text{g/mL}$)	25	316.5 \pm 42.61	302.0 \pm 30.57	25	3.0 \pm 2.28	3.0 \pm 1.54
¹Simao et al, 2012	Soybeans,50 mg/d	CRP (mg/L)	16	7.48 \pm 2.73	6.23 \pm 2.46	15	4.41 \pm 1.89	3.52 \pm 1.34

w14. Subgroup analyses

Example: We conducted a systematic review and meta-analysis to investigate the associations between phytoestrogen supplementation and body weight and the main parameters of body composition in postmenopausal women. Due to high heterogeneity in the main analyses we decided to explore which study/participant characteristics might be the source of heterogeneity. We stratified the analyses by the primary goal of the study, health status, median years since menopause, route of phytoestrogen administration, type of phytoestrogens, duration of the intervention, number of study participants, study location, and study quality. Each of the parameters we used for stratification had a biological basis. For example, we stratified by median years since onset of menopause because closer to onset women are more prone to gain weight than if they are further from menopause. Also, location, in this case, might play an important role. Soy, which is a cornerstone of traditional Asian diets, is rich in phytoestrogens, while in western countries soy is not that popular. Therefore, we stratified by location, which may act as a proxy for lifetime exposure to soy intake (which may play a role in observed associations).

Although I^2 values varied across subgroup analysis, using meta-regression it was not possible to explain the observed heterogeneity made by any of the investigated parameters. When stratified by median number of trial participants, in small RCTs with median number of participants ≤ 66 phytoestrogen supplementation was associated with significant decrease in body weight [pooled mean difference of changes -0.49Kg (95%CI: -0.87 to -0.11)](Table below).

Subgroups by Study Characteristics		Number of studies	¹ Difference, Mean (95 % CI)	² I ² for heterogeneity	³ P value for heterogeneity
Phytoestrogen use and mean body weight change					
^a Primary study goal of the RCT	Body composition	7	-0.04 (-0.68; 0.60)	68.7%	0.59
	Other	11	-0.21 (-0.64; 0.23)	62.3%	
^b Median years since menopause onset	≤4.1 years	4	-0.23 (-0.6; 0.14)	16.5%	0.87
	>4.1 years	8	0.07 (-0.51; 0.64)	68.2%	
	Unknown	6	-0.29 (-0.63; 0.04)	0%	
^c Route of administration	Tablet/capsule	10	-0.17 (-0.82; 0.47)	76.4%	0.52
	Diet	8	-0.17 (-0.42; 0.07)	0%	
^d Intervention type	Soy products	6	-0.49 (-1.21; 0.23)	65.4%	0.83
	Isoflavone mix	10	-0.24(-0.46; -0.01)	0%	
	Daidzein	2	0.92 (0.24; 1.59)	55%	
^e Median number of study participants	≤66 women	9	-0.49 (-0.87; -0.11)	0%	0.22
	>66 women	9	0.11 (-0.31; 0.52)	75.2%	
^f Intervention duration	≤24 weeks	15	-0.10 (-0.51; 0.30)	69.5%	0.9
	> 24weeks	3	-0.32 (-1.02; 0.37)	53.2%	
^g Location	Asia	7	0.22 (-0.25; 0.69)	76.8%	0.48
	Europe	4	-0.35 (-0.78; 0.07)	11%	
	North America	4	-0.6 (-1.44; 0.24)	0%	
	South America	3	-0.48 (-1.13; 0.17)	0%	
^h Risk of bias	High	2	-0.85 (-1.93; 0.22)	0%	0.39
	Low to medium	16	-0.09 (-0.45; 0.27)	69.6%	

¹Mean difference refers to mean difference of changes between treatment groups in body weight and BMI (subjects using phytoestrogens as compared with the subjects from control/placebo group)

²I² for heterogeneity was calculated using fixed effects models

³P value for heterogeneity was evaluated using random effects meta-regression (in cases in which more than 8 studies were meta-analyzed).

^aSome of RCTs investigated the effect of phytoestrogens on body composition, while others investigated other outcomes but reported changes in anthropometric parameters at baseline and at the end of the studies

^bMedian years since menopause, number of years since menopause onset, unknown: no information

^cRoute of administration includes tablets/capsules use and other routes of administration (shake, powder, flower)

^dType of phytoestrogens includes use of soy derived isoflavones/soy protein + isoflavones, extracts of soy isoflavones/isoflavone mixture, daidzein/genistein supplements

^eNumber of study participants: median number of participants calculated for each outcome separately

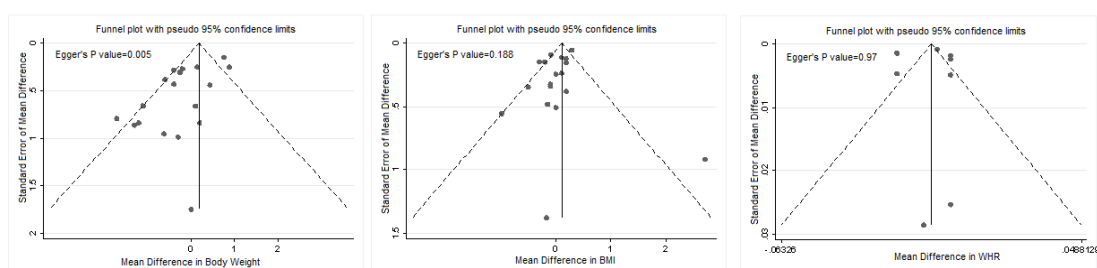
^fIntervention duration: median intervention duration was calculated for each outcome separately

^gLocation refers to study location, studies done in Asian ground and the other location (studies done in Europe, the Americas, and Australia)

^hStudies are judged to be at lower high risk of bias based on criteria to evaluate random sequence generation, allocation concealment, blinding of participants/personnel and outcome assessment, incomplete outcome data and selective reporting

w15. Funnel plots for RCTs included in the main analysis

Example: A recent meta-analysis that investigated the associations between phytoestrogen supplementation and body weight and the main parameters of body composition in postmenopausal women. In the funnel plots, the dotted lines show 95% confidence intervals around the overall summary estimate calculated using a random effects model, while each dot represents an RCT included in the analysis. In the first funnel plot, dots are asymmetrically oriented indicating presence of publication bias. This is in line with Egger's p value of 0.005, which favours existence of publication bias. In comparison, the dots are less asymmetrical in middle funnel plot, while in the third funnel plot they appear to be symmetrical. Also, Egger's p values are not significant, which indicates minimal possibility of publication bias for RCTs investigating body mass index and waist-to-hip ratio (**Appendix XIII**)¹⁰.



*First funnel plot refers to body weight, second to Body mass index, and third to waist circumference.

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