Lead Article

Effects of hesperidin consumption on cardiovascular risk biomarkers: a systematic review of animal studies and human randomized clinical trials

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Context: *The cardioprotective effects of the flavonoid hesperidin, which is present in citrus products, are controversial and unclear. This systematic review was conducted in accordance with the PRISMA 2015 guidelines.* ***Objective:*** *To evaluate the current evidence from animal and human clinical studies and thus determine whether the consumption of hesperidin exerts beneficial effects on cardiovascular risk factors.* ***Data sources:*** *PICOS (Population, Intervention, Comparison, Outcome, and Study Design) criteria defined the research question. Searches of the PubMed and Cochrane Plus databases were conducted and studies that met the inclusion criteria and were published in English in the last 15 years were included.* ***Data extraction:*** *The first author, year of publication, study design, characteristics of animals and humans, intervention groups, dose of hesperidin, route of administration, duration of the intervention, cardiovascular risk biomarkers assessed, and results observed were extracted from the included articles.* ***Results:*** *A total of 12 animal studies and 11 randomized clinical trials met the inclusion criteria. In the animal studies, the glucose, total and LDL cholesterol, and triglyceride levels decreased with chronic flavonoid consumption. In the human studies, endothelial function improved with flavonoid consumption, whereas no conclusive results were observed for the other biomarkers.* ***Conclusions:*** *Animal studies have revealed that hesperidin and hesperetin consumption reduces glucose levels and various lipid profile parameters. However, a definitive conclusion cannot be drawn from the existing human clinical trials. Further research is needed to confirm whether the findings observed in animal models can also be observed in humans.* ***Systematic Review Registration:*** *Prospero registration number CRD42018088942.*

INTRODUCTION

Cardiovascular diseases (CVDs) constitute the main cause of mortality throughout the world.[1] The latest statistical data from the World Health Organization showed that ischemic heart disease and stroke caused 15 million deaths in 2015 worldwide.[1]

Currently, there is a growing interest in identifying new bioactive compounds with healthy effects on CVDs, which can then be used to develop functional

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foods, and phenolic compounds have gained much interest in this field of research. Polyphenols are secondary metabolites of plants, and more than 8 000 different types exist, which can be classified into different groups depending on the number of phenolic rings they contain and the type of substituent attached to the rings.[2] Polyphenols are divided into two large families: flavonoids and non-flavonoids. Flavonoids are the most abundant type in plants, and the main subclasses include flavonols, flavones, isoflavones, flavanones, anthocyanidins, flavan-3-ols, and dihydrochalcones.[2] Flavonoids can be found in many commonly consumed fruits and vegetables, and numerous studies have shown their benefits for the prevention and treatment of different pathologies.[3–5] In recent years, citrus flavonoids, which are present in different citrus fruits, particularly in orange juice, have gained the attention of the food industry because they may exert beneficial effects on different cardiovascular risk factors (CVRFs)[6] and because orange juice is one of the most consumed beverages throughout the world.[7] In European adults, the mean flavonoid intake is 428 mg/day.[8]

The main citrus flavonoid of orange fruit and orange juice is hesperidin, which is found in greater quantities in the peel and represents 90% of citrus flavonoids.[9] Hesperidin (hesperetin-7-O-rutinoside) is a flavanone glycoside and the dietary form of the aglycone hesperitin.[6] Normally, the absorption of flavonoid glycosides such as hesperidin occurs in epithelial cells in the small intestine and is facilitated by the enzymes lactase phlorizin hydrolase or cytosolic β-glucosidase, resulting in the separation of the aglycone and its transportation into the bloodstream.[2] Then, the metabolites are transported to the liver for phase II metabolism, and they can be recycled by the enterohepatic recirculation in the small intestine. However, bioavailability studies show that only 30% of hesperetin metabolites are absorbed in the small intestine and the other 70% are absorbed in the colon,[2] via microbiota and alpha-rhamnosidase activity,[10] where the hesperidin is converted to glucuronides. In-vitro studies have revealed that hesperidin stimulates the production of nitric oxide (NO) in endothelial cells,[11,12] inhibits the secretion of endothelin-1[12] and inhibits platelet activity by inhibiting the activities of specific phospholipases and cyclooxygenase-1.[13] Animal studies have shown that hesperidin exhibits antioxidant capacity and endothelial protection against reactive oxygen species in spontaneously hypertensive rats, and improves hyperlipidemia and hyperglycemia in diabetic rats.[14] Conversely, other animal studies have not found that hesperidin exerts beneficial effects on glucose or insulin levels, lipid profile, or blood pressure.[15,16] In contrast, several observational studies have shown that citrus fruit consumption is associated with a lower risk of acute coronary events.[17,18] However, the findings from human randomized clinical trials (RCTs) are not consistent: some studies have found that daily consumption of orange juice decreases systolic blood pressure (SBP) and diastolic blood pressure (DBP),[19] and increases the total plasma antioxidant capacity or decreases lipid peroxidation,[20] but others have not reported any beneficial effects on blood pressure or the lipid profile after hesperidin consumption.[21,22] To the best of our knowledge, the current scientific evidence on the effects of hesperidin on cardiovascular risk biomarkers obtained from animal studies and human RCTs has not been systematically reviewed, and thus, no conclusive remarks can be drawn.

Therefore, the present systematic review aimed to determine whether hesperidin consumption might exert beneficial effects on cardiovascular risk biomarkers. The objective was to summarize and evaluate the current scientific evidence from animal studies and human RCTs to determine the effects of hesperidin on cardiovascular risk biomarkers.

METHODS

This systematic review was conducted according to the PRISMA 2015[23] (Preferred Reporting Items for Systematic reviews and Meta-Analysis) guidelines and was registered with PROSPERO on February 20, 2018, under the ID number CRD42018088942. The protocol can be accessed at http://www.crd.york.ac.uk/PROSPERO/display_record.php? ID=CRD42018088942.

Eligibility criteria

Animal studies and RCTs were eligible for the systematic review in accordance with the review's PICOS criteria. The complete PICOS criteria for inclusion and exclusion of studies are described in Table 1.

Information sources, search strategy, and study selection

A literature search of the PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and Cochrane Plus (www.bibliotecaco-gov/pubmed) databases was performed using medical subject headings (MeSH). The complete search strategy is shown in Table 2. The literature search was restricted to English-language articles published between January 2003 and January 2018.

To ensure the accurate identification of eligible studies, a two-step selection process was used. To confirm the eligibility of the included articles, the titles and

Table 1 **PICOS criteria for inclusion and exclusion of studies**

Parameter	Inclusion criteria	Exclusion criteria
For the animal studies		
Participants	Rats or mice with at least one CVRF (obesity, dyslipidemia, hypertension, diabetes, or metabolic syndrome)	Studies performed on animal models that were not rats or mice
Intervention	Some type of intervention based on hesperidin	Combination of different classes of phenolic compounds (other than citrus flavonoids) and combination with other nutrients, components, or drugs (vitamin C, caffeine, or hypertension drugs)
Comparisons	Different doses of hesperidin and/or hesperidin consumption and non-consumption	
Outcomes	Studies that assessed the effects of hesperidin on biomarkers or risk factors related to CVDs: anthropometric parameters, vascular parameters, glucose and insulin levels, lipid profile and coagulation, inflammation and oxidation biomarkers	
Study design	Randomized and non-randomized, acute and chronic follow-up, published in English	Studies published before January 2003 and in any language other than English
For the RCTs		
Participants	Humans of all races, ages, and genders with at least one CVRF (obesity, dyslipidemia, hypertension, diabetes, or metabolic syndrome)	Humans with no CVRF
Intervention	Some type of nutritional intervention based on the consumption of hesperidin from food, drink, or supplement	Combination of different classes of phenolic compounds (other than citrus flavonoids) and combination with other nutrients, components, or drugs (vitamin C, caffeine, or hypertension drugs)
Comparisons	Different doses of hesperidin and/or hesperidin consumption and non-consumption	
Outcomes	Studies that assessed the effects of hesperidin consumption on biomarkers or risk factors related to CVD: anthropometric parameters, vascular parameters, glucose and insulin levels, lipid profile and coagulation, inflammation and oxidation biomarkers	
Study design	Randomized controlled clinical trials, parallel and crossover design, acute and chronic follow-up, published in English	Reviews, expert opinion, comments, letter to editor, case reports, conference reports, observational studies, animal studies, and studies published before January 2003 and in any language other than English

Abbreviations: CVDs, cardiovascular diseases; CVRF, cardiovascular risk factor; RCTs, randomized clinical trials.

abstracts of the studies identified using the search strategy were screened independently by two authors (LP-P and JC). The full text of the potentially eligible studies was then retrieved and independently assessed for eligibility by the same two authors. Any disagreement between the authors over the eligibility of a study was resolved through discussion with a third author (LC-P).

Data collection and extraction

From the total number of articles identified by assigning appropriate MeSH terms, any duplicate articles within and between the databases were removed. The remaining articles were assessed primarily according to their title and abstract, and then according to their full text, and those studies that did not meet the eligibility criteria were removed.

The following data were extracted from the included animal studies: first author, year of publication, study design, characteristics of the animals, intervention groups, dose of hesperidin, route of administration, duration of the intervention, cardiovascular risk (CVR) biomarkers assessed, and results observed.

The following data were extracted from the RCTs: first author, year of publication, study population, population age and health status, characteristics of the nutritional intervention, dose of hesperidin, consumption matrix, duration of the intervention, method used to confirm compliance with the intervention, CVR biomarkers assessed, and results observed.

Study quality and risk of bias in the individual studies

Assessments of the quality and possible risks of bias in each RCT included in the present systematic review were performed using Review Manager software (RevMan; version 5.3), a tool provided by the Cochrane Collaboration. The following items were included in the assessments: random sequence generation, allocation concealment, blinding of participants and personnel,

Table 2 **Search strategy and MeSH terms used**

For the animal studies	For the RCTs
Search strategy: -Electronic databases: PubMed and Cochrane Plus -Publication dates: January 2003 – January 2018 -Species: Other animals	Search strategy: -Electronic databases: PubMed and Cochrane Plus -Publication dates: January 2003 – January 2018 -Species: Humans
MeSH terms: hesperidin hesperetin and blood pressure endothelial function blood cholesterol high density lipoprotein low density lipoprotein apolipoprotein A1 apolipoprotein B100 triglycerides plasma no esterified reactive protein glucose insulin resistance diabetes C-reactive protein IL-6 IL-18 nitrates and nitrites platelet aggregation endothelin soluble intercellular adhesion molecule-1 soluble vascular cell adhesion molecule-1 E-selection serum amyloid A oxidized low density lipoprotein urinary creatinine oxidative stress nitric oxide homocysteine nitrotyrosine plasminogen activator inhibitor-1 von Willebrand factor fibrinogen body mass index body weight obesity overweight	MeSH terms: orange juice orange polyphenols citrus flavonoids citrus flavanones hesperidin hesperetin and blood pressure hypertension endothelial function blood cholesterol high density lipoprotein low density lipoprotein apolipoprotein A1 apolipoprotein B100 triglycerides plasma no esterified reactive protein glucose insulin resistance diabetes IL-6 IL-18 nitrates and nitrites platelet aggregation endothelin soluble intercellular adhesion molecule-1 soluble vascular cell adhesion molecule-1 E-selection serum amyloid A oxidized low density lipoprotein urinary creatinine oxidative stress nitric oxide homocysteine nitrotyrosine plasminogen activator inhibitor-1 von Willebrand factor fibrinogen body mass index body weight obesity overweight atherosclerosis cardiovascular risk factors

Abbreviation: RCTs, randomized clinical trials.

blinding of outcome assessments, incomplete outcome data, selective reporting, and other biases. The risk of bias in each study was classified as "low," "unclear," or "high." Two authors (LP-P and JC) evaluated the risk of bias in the RCTs, and any disagreement between them over the risk of bias of a study was resolved through discussion with a third author (LC-P).

RESULTS

Animal studies

Study selection. A total of 698 articles were identified from the two databases (643 in PubMed and 55 in Cochrane Plus). Of these, 367 duplicate articles were

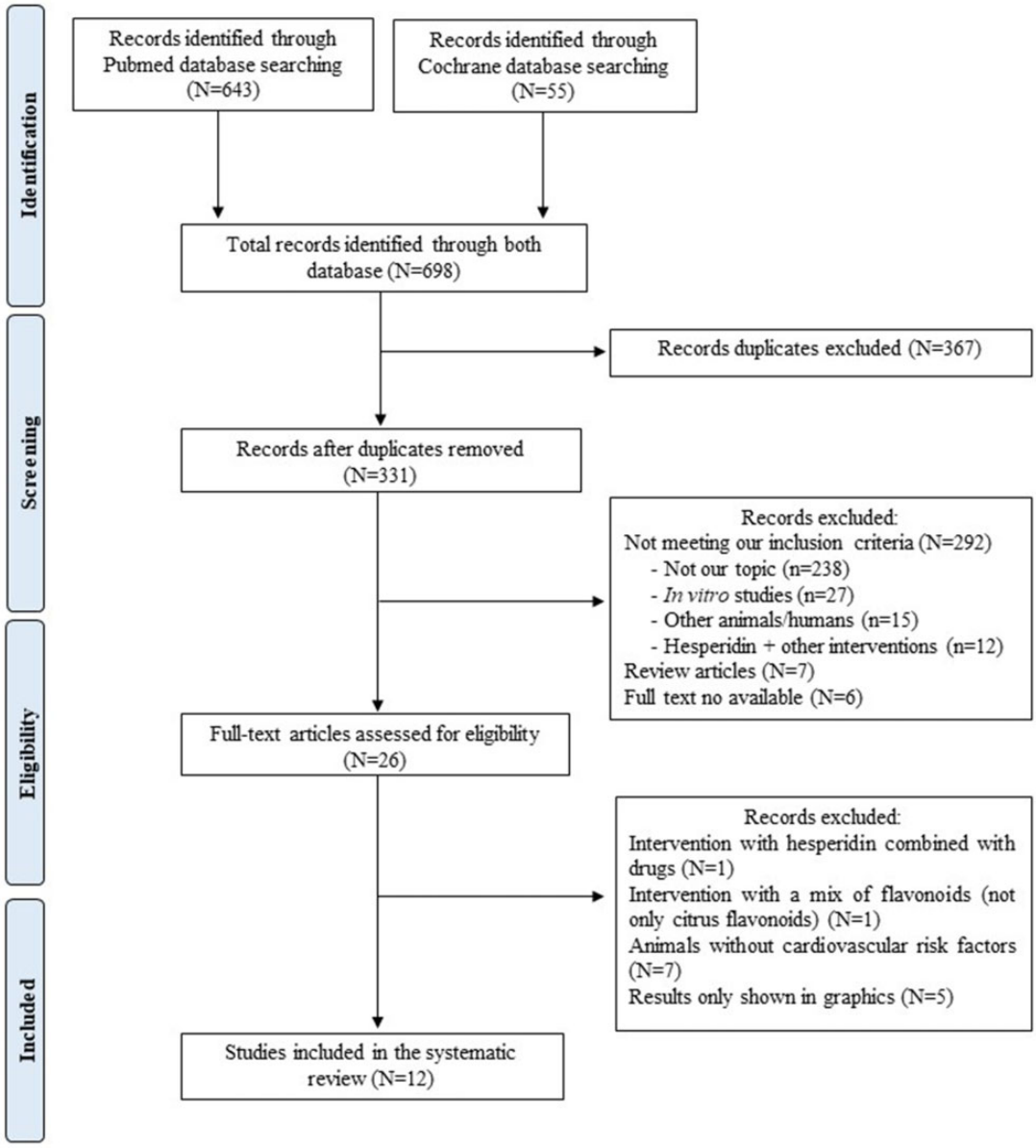

Figure 1 **Flow diagram of the literature search process for animal studies.**

removed and 292 of the remaining 331 articles were excluded because they did not meet the inclusion criteria, 7 were excluded because they were review articles, and 6 were excluded because no full text was available. As a result, 12 articles were included in the systematic review. Figure 1 shows the study selection process for the animal studies included in the review.

Study characteristics. Table 3 shows the general characteristics of the 12 animal studies included in the systematic review. Further details of each study are presented in Table S1 in the Supporting Information online. The 12 studies included in the systematic review were controlled animal studies involving an intervention group that was administered flavanone and a control group that was not administered flavanone. In 9 of the studies, hesperidin was orally administered,[15,21,22,24–29] while in 2 of the remaining 3 studies hesperidin was administered by gavage,[30,31] and in the other study hesperidin was administered intravenously.[32] The doses of hesperidin ranged from 5 mg/kg of body weight/day to 200 mg/kg of body weight/day in 10 studies and from 0.08% to 4.60% of the total calorie intake in the other 2 studies. The duration of the intervention ranged from 7 days to 24 weeks. All the animals had at least one CVRF, such as hypertension, myocardial ischemia, systemic inflammation, hypercholesterolemia, and type 2 diabetes. The sample size ranged from 4 to 16 animals in each group, and of the 12 studies, 8 were performed on rats and 4 on mice.

Table 3 **Characteristics and results of the animal studies included in the systematic review (n = 12)**

Author, year, reference	Experimental animal	Groups (n)	Dose of flavanone	Route	Duration	Results													
						Anthropometric parameters			Vascular parameters		Glucose and insulin levels		Lipid profile				INFL. BIOM	OXID. BIOM
						BW	WG	VF	SBP	DBP	GL	INS	TC	LDL-c	HDL-c	TG	IL-6	NO
Iskender et al (2017)[24]	Type 2 diabetic rats	Control group (n=10) vs Hesperidin group (n=10)	0 mg vs 100 mg/kg BW/d	Orally	15 d	NS	–	–	–	–	↓	–	–	–	–	–	–	–
Dobiaš et al (2016)[25]	Spontaneously hypertensive rats	Control group (n=13) vs Hesperidin group (n=13)	0 mg vs 50 mg/kg BW/d	Orally	4 wk	NS	–	–	NS	–	–	–	–	–	–	–	–	–
Ferreira et al (2016)[15]	Mice with systemic inflammation	Control group (n=10) vs Hesperidin group (n=10)	0 mg vs 100 mg/kg BW/d	Orally	4 wk	NS	NS	NS	–	–	NS	–	NS	↓	NS	NS	↓	–
Jia et al (2015)[31]	Type 2 diabetic mice	Control group (n=10) vs Neohesperidin group (n=10)	0 mg vs 50 mg/kg BW/d	By gavage	6 wk	NS	–	–	–	–	↓	–	↓	–	–	↓	–	–
Yamamoto et al (2013)[32]	Hypertensive rats	Control group (n=4) vs Hesperetin group (n=4) vs Hesperetin-7-O-β-D-glucuronide group (n=4) vs Hesperetin-3′-O-β-D-glucuronide group (n=4)	0 mg vs 5 mg/kg BW/d vs 5 mg/kg BW/d vs 5 mg/kg BW/d	Intravenous	3 min (acute study)	–	–	–	↓ with hesperetin; ↓ with HESPT7G	NS	–	–	–	–	–	–	–	–
Kumar et al (2012)[30]	Type 2 diabetic rats	Control group (n=16) vs Hesperetin group (n=16)	0 mg vs 200 mg/kg BW/d	By gavage	24 wk	–	–	–	–	–	↓	–	–	–	–	–	–	–

(continued)

Table 3 **Continued**

Author, year, reference	Experimental animal	Groups (n)	Dose of flavanone	Route	Duration	Results													
						Anthropometric parameters			Vascular parameters		Glucose and insulin levels		Lipid profile				INFL. BIOM	OXID. BIOM
						BW	WG	VF	SBP	DBP	GL	INS	TC	LDL-c	HDL-c	TG	IL-6	NO
Mahmoud et al (2012)[26]	Type 2 diabetic rats	Control group (n=6) vs Hesperidin group (n=6)	0 mg 50 mg/kg BW/d	Orally	30 d	–	–	–	–	–	↓	↑	–	–	–	–	–	↓
Selvaraj and Pugalendi (2012)[22]	Rats with myocardial ischemia	Control group (n=6) vs Hesperidin group (n=6)	0 mg 200 mg/kg BW/d	Orally	7 d	–	–	–	–	–	–	–	↓	↓	↑	↓	–	–
Wang et al (2011)[16]	Hypercholesterolemic rats	Control group (n=15) vs Hesperidin (n=15)	0% 0.08% TCD/d	Orally	12 wk	NS	–	–	NS	NS	–	–	–	–	–	–	–	–
Akiyama et al (2009)[27]	Type 2 diabetic rats	Control group (n=6) vs Hesperidin group 1 (n=6) vs Hesperidin group 2 (n=6)	0% TCD/d 1% TCD/d 4.6% TCD/d	Orally	4 wk	↓ with 4.6%	–	–	–	–	↓ with 4.6%	↓ with 4.6%	↓	–	–	↓	–	–
Jung et al (2006)[28]	Type 2 diabetic mice	Control group (n=10) vs Hesperidin group (n=10)	0 mg 200 mg/kg BW/d	Orally	5 wk	–	–	–	–	–	↓	–	↓	–	NS	↓	–	–
Jung et al (2004)[29]	Type 2 diabetic mice	Control group (n=10) vs Hesperidin group (n=10)	0 mg 200 mg/kg BW/d	Orally	5 wk	–	–	–	–	–	–	↑	–	–	–	–	–	–

-, parameter not evaluated; ↓, significant decrease in intervention group vs control group; ↑, significant increase in intervention group vs control group.

Abbreviations: BW, body weight; DBP, diastolic blood pressure; GL, glucose; HDL-c, high density lipoprotein; IL-6, interleukin-6; INFL. BIOM, inflammation biomarkers; INS, insulin; LDL-c, low-density lipoprotein; NO, nitric oxide; NS, no significant differences between intervention group and control group; OXID. BIOM, oxidation biomarkers; SBP, systolic blood pressure; TC, total cholesterol; TCD, total calorie diet; TG, triglycerides; VF, visceral fat; WG, weight gain.

Results for anthropometric parameters. The effect of hesperidin consumption on body weight was evaluated in 6 studies.[15,21,24,25,27,30] Of these, 4 studies reported no significant changes,[15,16,24,25] 1 study reported a significant decrease,[27] and 1 study did not specify the outcome.[30] Akiyama et al[27] reported that the administration of a daily oral dose of hesperetin of 4.60% of total calorie intake to type 2 diabetic rats for 4 weeks prevented the weight gain, of 13.56 g, observed in the control group. In another study that examined the effect of hesperidin consumption on visceral fat,[15] no significant changes were observed.

Results for vascular parameters. The effect of hesperidin on SBP was evaluated in 3 studies[21,25,32]: 2 of these studies reported no significant changes[21,25] and 1 study reported a significant decrease in SBP.[32] Yamamoto et al[32] reported that intravenous administration of an acute dose of 5 mg/kg of body weight of hesperetin to hypertensive rats significantly decreased SBP by 9.90 ± 1.70 mmHg, compared with the control group. The same study[32] also reported that an acute dose of 5 mg/kg of body weight of hesperetin-7-O-β-D-glucuronide significantly decreased SBP by 8.70 ± 0.80 mmHg, compared with the control group. The effect of hesperidin on DBP was evaluated in 2 studies,[21,32] but no significant changes were observed.

Results for glucose and insulin levels. The effect of hesperidin on blood glucose was evaluated in 7 studies.[15,24,26–28,30,31] Six of these studies reported decreases in blood glucose[24,26–28,30,31] and 1 study found no significant changes.[15] Iskender et al[24] reported that the oral consumption of 100 mg/kg of body weight/day of hesperidin for 15 days significantly lowered blood glucose levels in type 2 diabetic rats by 9.25 mmol/L, compared with the control group. Jia et al[31] observed that the consumption of 50 mg/kg of body weight/day of neohesperidin (derived from hesperidin) by gavage for 6 weeks significantly lowered blood glucose levels in type 2 diabetic mice by 7.73 mmol/L, compared with the control group. Kumar et al[30] found that the consumption of 200 mg/kg of body weight/day of hesperetin by gavage for 24 weeks significantly lowered blood glucose levels in type 2 diabetic rats by 5.99 mmol/L, compared with the control group. Mahmoud et al[26] detected significant reductions – of 9.49 mmol/L – in the blood glucose levels of type 2 diabetic rats after oral consumption of 50 mg/kg of body weight/day of hesperidin for 30 days, compared with the control group. Akiyama et al[27] found that daily consumption of hesperetin at a dose of 4.60% of total calorie intake for 4 weeks significantly lowered blood glucose levels in type 2 diabetic rats by 1.61 mmol/L, compared with the control group. In addition, Jung et al[28] reported that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 5 weeks significantly lowered blood glucose levels in type 2 diabetic mice by 7.84 mmol/L, compared with the control group.

The effect of hesperidin on serum insulin levels was evaluated in 3 studies,[26,27,29] of which 2 reported significant increases in insulin levels[26,29] and 1 reported a significant decrease.[27] Mahmoud et al[26] reported that the oral consumption of 50 mg/kg of body weight/day of hesperidin for 30 days significantly raised insulin levels in type 2 diabetic rats by 6.05 μU/mL, compared with the control group. Jung et al[29] found a significant increase of 18.13 μU/mL in the insulin levels of type 2 diabetic mice after 5 weeks of oral consumption of 200 mg/kg of body weight/day of hesperidin, compared with the control group. Moreover, Akiyama et al[27] reported that oral daily consumption of hesperidin at a dose of 4.60% of total calorie intake for 5 weeks of intervention significantly lowered insulin levels in type 2 diabetic rats by 90.64 μU/mL, compared with the control group.

Results for lipid profile. The effect of hesperidin consumption on total cholesterol (TC) levels was evaluated in 4 studies.[15,22,27,28] Of these, 3 reported significant decreases in TC levels[22,27,28] and 1 study found no significant changes.[15] Selvaraj and Pugalendi[22] observed that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 7 days significantly lowered TC levels in rats with myocardial ischemia by 0.40 mmol/L, compared with the control group. Akiyama et al[27] noted that daily consumption of hesperetin at a dose of 1% and 4.60% of total calorie intake for 4 weeks significantly lowered TC levels in type 2 diabetic rats by 1.71 mmol/L and 2.51 mmol/L, respectively, compared with the control group. Moreover, Jung et al[28] reported that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 5 weeks significantly lowered TC levels in type 2 diabetic mice by 0.81 mmol/L, compared with the control group.

The effect of hesperidin consumption on high-density lipoprotein cholesterol (HDL-c) levels was evaluated in 3 studies.[15,22,28] Two of these studies reported no significant changes in HDL-c[15,28] and the other study reported a significant increase.[22] Selvaraj and Pugalendi[22] reported that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 7 days significantly increased HDL-c levels in rats with myocardial ischemia by 0.34 mmol/L, compared with the control group.

The effect of hesperidin consumption on low-density lipoprotein cholesterol (LDL-c) levels was assessed

in 2 studies,[15,22] and significant decreases were observed in both studies. Ferreira et al[15] observed that the oral consumption of 100 mg/kg of body weight/day of hesperidin for 15 days significantly lowered LDL-c levels in mice with systemic inflammation by 0.29 mmol/L, compared with the control group. In addition, Selvaraj and Pugalendi[22] observed that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 7 days significantly decreased LDL-c by 0.67 mmol/L, compared with the control group.

The effect of hesperidin consumption on triglyceride (TG) levels was evaluated in 5 studies.[15,22,27,28,31] Four of these studies reported significant decreases[22,27,28,31] and the other study reported no significant changes.[15] Jia et al[31] noted that the consumption of 50 mg/kg of body weight/day of neohesperidin by gavage for 6 weeks significantly lowered TG levels in type 2 diabetic mice by 2.05 mmol/L, compared with the control group. In rats with myocardial ischemia, Selvaraj and Pugalendi[22] observed that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 7 days significantly lowered TG levels by 0.18 mmol/L, compared with the control group. Akiyama et al[27] noted that daily consumption of hesperetin at a dose of 1% and 4.60% of total calorie intake for 4 weeks lowered TG levels in type 2 diabetic rats by 0.66 mol/L and 0.91 mmol/L, respectively, compared with the control group. Lastly, Jung et al[28] stated that the oral consumption of 200 mg/kg of body weight/day of hesperidin for 5 weeks of intervention lowered TG levels in type 2 diabetic mice by 1.74 mmol/L, compared with the control group.

Results for inflammation biomarkers. The effect of hesperidin on interleukin-6 (IL-6) levels was evaluated in a study by Ferreira et al.[15] Using a mouse model of systemic inflammation, this study reported a significant decrease of 58.64 pg/mL after the oral consumption of 100 mg/kg of body weight/day of hesperidin for 4 weeks, compared with the control group.

Results for oxidation biomarkers. The effect of hesperidin on nitric oxide levels was evaluated in a study by Mahmoud et al.[26] The study reported a significant decrease of 5.08 mg/dL after the oral consumption of 50 mg/of body weight/day of hesperidin for 30 days in type 2 diabetic rats, compared with the control group.

Human randomized controlled trials

Study selection. A total of 1917 articles were identified from the searches of the two databases (1 495 in PubMed and 422 in Cochrane Plus). Of these, 1 486 duplicate articles were removed and 393 were excluded because they did not meet the inclusion criteria. Thus, 11 articles were included in the systematic review. Figure 2 shows the study selection process for the RCTs included in the review.

Study characteristics. Tables 4 to 6 show the characteristics of the RCT studies included in this systematic review. Further details of each study are presented in Table S2 in the Supporting Information online. The 11 studies included in this review were RCTs involving some type of nutritional intervention. In fact, the interventions in 3 of the 11 included RCTs consisted of supplementation with a placebo capsule or a hesperidin capsule,[11,33,34] whereas those in the 3 other studies consisted of the administration of a control drink (CD) or orange juice (OJ).[35–37] In addition, the interventions in 2 other studies involved the consumption of different drinks with different hesperidin concentrations,[38,39] whereas those in 2 and 1 of the remaining RCTs consisted of no product intervention vs OJ administration[40,41] and supplementation with a placebo or hesperidin capsule or consumption of OJ, respectively.[42]

Four of the studies comprised a parallel design,[33,34,40,41] and the other seven comprised crossover designs.[11,35–39,42] Ten of the included RCTs involved a long-term follow-up, and one of these also involved a short-term follow-up. The other RCT involved only a short-term follow-up. The duration of the intervention in the long-term studies ranged from 1.5 to 13 weeks, and the duration in the short-term studies ranged from 4 to 5 hours. Nine of the studies were conducted with European populations, and the other 2 investigated South American populations. The sample sizes ranged from 22 to 194 subjects, and the ages of the subjects ranged from 18 to 69 years. All the subjects had at least one CVRF, such as dyslipidemia, overweight, obesity, and/or metabolic syndrome. The methods used to confirm intervention compliance involved keeping 3- or 5-day food records, maintaining 24-hour dietary records, returning all used and unused capsule boxes, and self-reporting.

Assessment of the quality and risk of bias. The risk of bias in each individual RCT is detailed in Figure 3. Six of the 11 RCTs used an adequate random sequence generator; 3 studies incorporated adequate allocation concealment; 5 studies performed adequate blinding of the participants, personnel, and outcome assessment; 9 studies presented completed data; and 6 studies presented their study protocol with all the reported outcomes. Regarding other types of bias, potential conflicts of interest were considered, and 8 studies reported a lack of conflicts of interest.

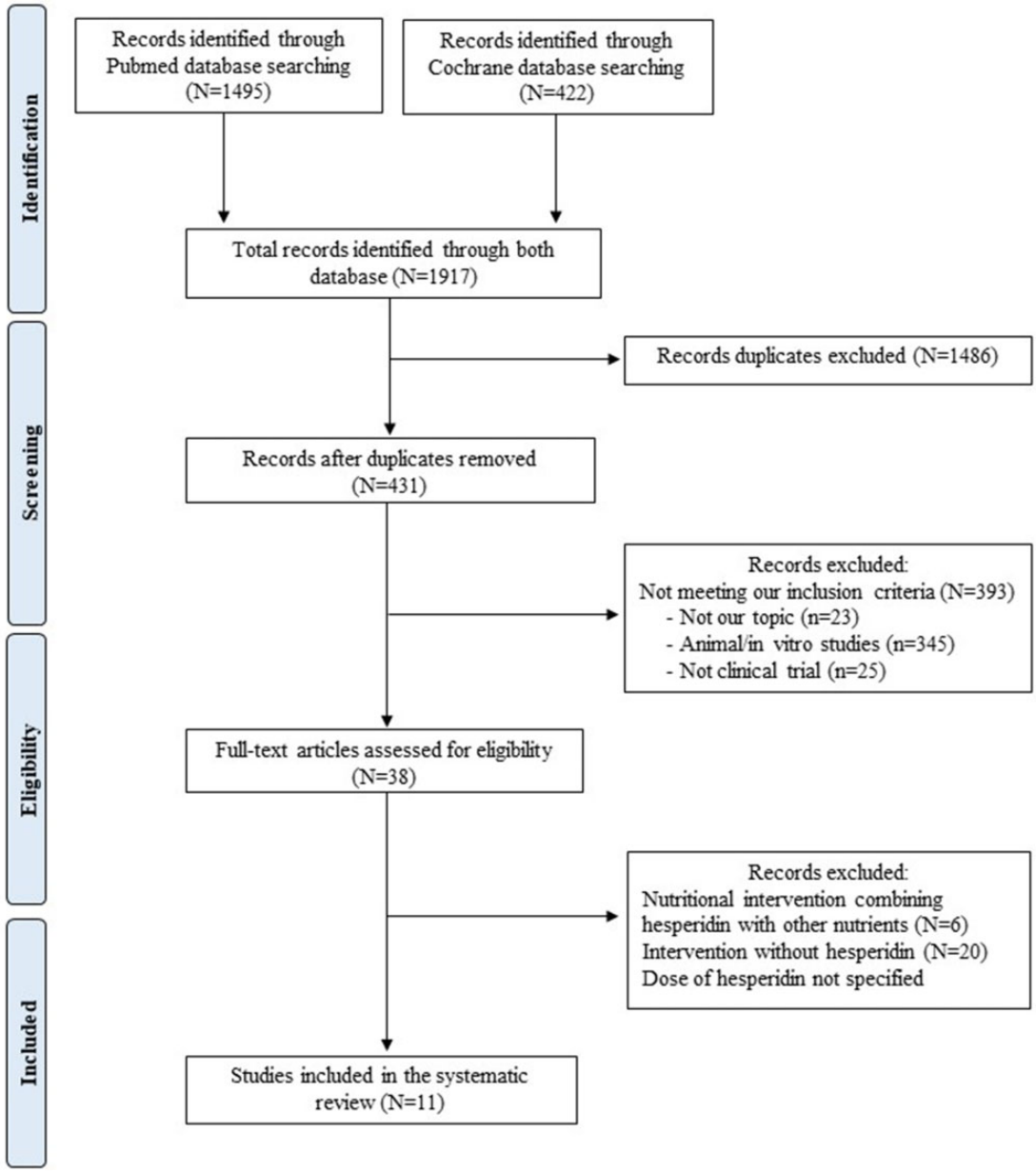

Figure 2 **Flow diagram of the literature search process for randomized clinical trials.**

Effects of chronic hesperidin consumption on cardiovascular risk biomarkers.

Results for anthropometric parameters. The characteristics of the long-term RCTs included in this review in relation to anthropometric parameters are detailed in Table 4.

The effect of hesperidin consumption on body weight was evaluated in 3 studies.[34,39,40] Of these, 2 studies reported significant decreases,[39,40] and 1 study found no significant changes.[34] Rangel-Huerta et al[39] observed that the consumption of 237 mg/day or 582.50 mg/day of hesperidin in 500 mL/day of OJ for 12 weeks reduced the body weight of overweight or obese subjects by 1.30 kg and 1.80 kg, respectively, compared with basal levels. No differences between the different hesperidin concentrations were observed. Aptekmann and Cesar[40] noted that the consumption of 54.60 mg/day of hesperetin in 500 mL/day of OJ for 13 weeks of intervention significantly reduced the body weight of hypercholesterolemic subjects by 1 kg, compared with basal levels. No significant differences were observed between the intervention and control groups.

The effect of hesperidin consumption on the body mass index (BMI) was evaluated in 5 studies[11,34,39–41]: 3 of these studies reported no significant changes[11,34,41] and 2 studies found significant decreases.[39,40] Rangel-Huerta et al[39] observed that the consumption of 237 mg/day and 582.50 mg/day of hesperidin in 500 mL/day

Table 4 **Characteristics of the long-term randomized clinical trials included in the systematic review with results for anthropometric and vascular parameters and glucose and insulin levels (n = 8)**

Author, year, reference	Sample size	Cardiovascular risk factors	Nutritional intervention				Results									
			Groups	Flavanone dose	Productdose	Duration	Anthropometric parameters			Vascular parameters				Glucose and insulin levels		
							BW, kg	BMI, kg/m2	BF, %	SBP, mm Hg	DBP, mm Hg	FMD, %	GTN, %	GLUC, mmol/L	INS, μU/mL	QUICKI
Salden et al (2016)[33]	n=65	Overweight or obesity	Placebo capsule vs Hesperidin capsule	0 mg/d 450 mg/d	500 mg/d 500 mg/d	6 wk	–	–	–	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	–	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)
Kean et al (2015)[38]	n=37	Overweight	Low hesperidin drink vs High hesperidin drink	64 mg/d 549 mg/d	500 mL/d 500 mL/d	8 wk	–	–	–	NS/NS (NS)	NS/NS (NS)	–	–	–	–	–
Constans et al (2015)[35]	n=25	Hypercholesterolemia	Control drink vs Orange juice (hesperidin)	0 mg/d 213 mg/d	600 mL/d 600 mL/d	4 wk	–	–	–	–	–	–	–	NS/NS(NS)	–	–
Rangel-Huerta et al (2015)[39]	n=100	Overweight or obesity	Orange juice (hesperidin) vs Enriched orange juice (hesperidin)	237 mg/d 582.5 mg/d	500 mL/d 500 mL/d	12 wk	↓1.3/↓1.8 (NS)	↓0.5/↓0.7 (NS)	–	↓4.00/NS (NS)	↓3.00/NS (NS)	–	–	↑0.3/↑0.2 (P <0.05)*	↓1.2/NS (P <0.05)*	–
Buscemi et al (2012)[37]	n=31	Metabolic syndrome	Control drink vs Orange juice (hesperidin)	0 mg/d 159.5 mg/d	500 mL/d 500 mL/d	1.5 wk	–	–	–	–	–	NS/↑2.2 (P <0.05)*	NS/NS(NS)	–	–	–
Rizza et al (2011)[11]	n=24	Metabolic syndrome	Placebo capsule vs Hesperidin capsule	0 mg/d 500 mg/d	1 capsule/d 1 capsule/d	3 wk	–	NS/NS (NS)	–	NS/NS (NS)	NS/NS (NS)	NS/↑2.5 (p=0.02)*	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)
Morand et al (2011)[42]	n=23	Overweight	Placebo capsule with control drink vs Hesperidin capsule with control drink vs Orange juice (hesperidin)	0 mg/d 292 mg/d 292 mg/d	1 capsule and 500 mL/d 1 capsule and 500 mL/d 500 mL/d	4 wk	–	–	–	NS/NS/NS (NS)	NS/↓5.3/↓4.5 (P <0.023)*	–	–	NS/NS/NS (NS)	NS/NS/NS (NS)	–
Demonty et al (2010)[34]	n=194	Overweight Hypercholesterolemia	Placebo capsule vs Hesperidin capsule	0 mg/d 800 mg/d	4 capsules/d 4 capsules/d	4 wk	NS/NS (NS)	NS/NS (NS)	–	–	–	–	–	–	–	–
Aptekmann and Cesar (2010)[40]	n=26	Overweight	No intervention vs Orange juice (hesperetin)	0 mg/d 54.6 mg/d	0 mL/d 500 mL/d	13 wk	↓1.8/↓1.0 (NS)	↓0.7/↓0.3 (NS)	↓5.5/↓4.3 (NS)	–	–	–	–	–	–	–
Cesar et al (2010)[41]	n=22	Hyperchole sterolemia	No intervention vs Orange juice (hesperetin)	0 mg/d 42 mg/d	0 mL/d 750 mL/d	8.5 wk	–	NS/NS (NS)	–	–	–	–	–	–	–	–

-, parameter not evaluated; ↓, significant decrease between basal and final value within each group; ↑, significant increase between basal and final value within each group; *, significant differences between groups.

Abbreviations: BF, body fat; BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; GLUC, glucose; GTN, glyceryl-nitrate dilation of the brachial artery; INS, insulin; NS, no significant differences between basal and final within each group; (NS); no significant differences between intervention group and control group; QUICKI, quantitative insulin-sensitivity check index; SBP, systolic blood pressure.

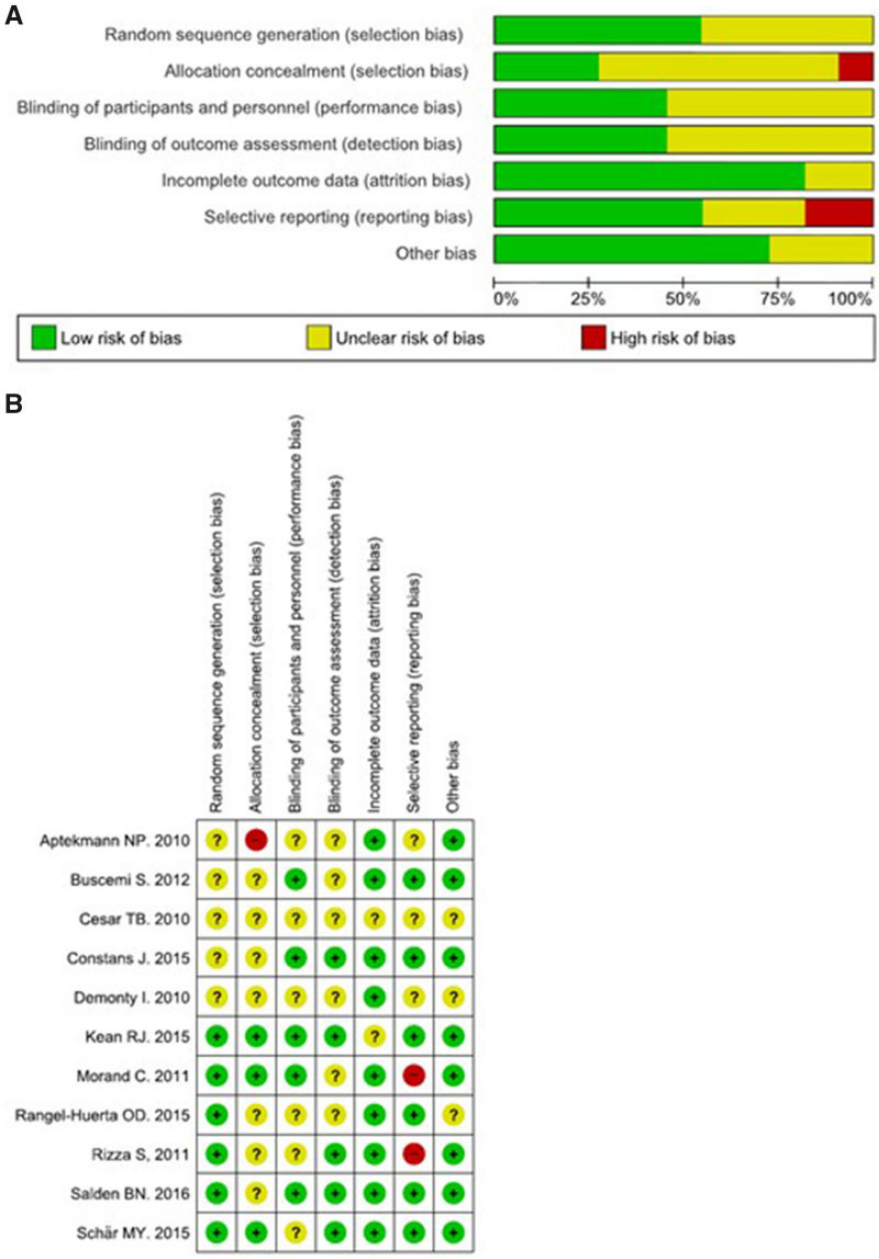

Figure 3 **Risk of bias graph (A) and summary (B) of the randomized clinical trials included.** + indicates a low risk of bias, - indicates a high risk of bias, and ? indicates an unclear risk.

of OJ for 12 weeks reduced the BMI of overweight or obese subjects by 0.50 kg/m^2 and 0.70 kg/m^2, respectively, compared with basal levels. No differences between the different hesperidin concentrations were observed. Aptekmann and Cesar[40] reported that the consumption of 54.60 mg/day of hesperetin in 500 mL/day of OJ significantly reduced the BMI of hypercholesterolemic subjects by 0.30 kg/m^2 after 13 weeks of intervention, compared with basal levels. No significant differences were observed between the intervention and control groups.

The effect of hesperidin consumption on body fat was evaluated in 1 study and a significant decrease was observed.[40] Specifically, Aptekmann and Cesar[40] reported that 54.60 mg/day of hesperetin in 500 mL/day of OJ significantly reduced the body fat of hypercholesterolemic subjects by 4.30% after 13 weeks of intervention, compared with basal levels. No significant differences were observed between the intervention and control groups.

Results for vascular parameters. The characteristics of the long-term RCTs included in this review in relation to vascular parameters are detailed in Table 4.

The effect of hesperidin consumption on SBP and DBP was evaluated in 5 studies.[11,33,38,39,42] Of these,

3 reported no significant changes[11,33,38] and significant decreases were detected in the other 2 studies.[39,42] Rangel Huerta et al[39] observed that the consumption of 237 mg/day of hesperidin for 12 weeks reduced the SBP and DBP of overweight or obese subjects by 4 mmHg and 3 mmHg, respectively, compared with basal levels. No significant differences were observed in a comparison with the group administered a lower concentration of hesperidin. Morand et al[42] reported that the consumption of 292 mg/day of hesperidin – in the form of pure hesperidin capsules or provided naturally with 500 mL/day of OJ for 4 weeks – reduced the DBP of overweight subjects by 5.30 mmHg and 4.50 mmHg, respectively, compared with basal levels. Significant differences were observed in a comparison with the control group.

The effect of hesperidin consumption on endothelial function was evaluated in 3 studies[11,33,36]: 2 of these studies reported significant increases[11,36] and the other study found no significant changes.[33] In subjects with metabolic syndrome, Buscemi et al[36] observed a significant increase in flow-mediated dilation (FMD) of 2.20% after 1.5 weeks of the consumption of 159.50 mg/day of hesperidin in 500 mL/day of OJ. Significant differences between the intervention group and the control group were observed. Similarly, in subjects with metabolic syndrome, Rizza et al[11] reported a significant increase in FMD of 2.48% after 3 weeks of the consumption of 500 mg/day of hesperidin in capsule form, and the differences between the intervention and control groups were significant.

Results for glucose and insulin levels. The characteristics of the long-term RCTs included in this review in relation to glucose and insulin levels are detailed in Table 4.

The effect of hesperidin consumption on plasma glucose levels was evaluated in 5 studies.[11,33,35,39,42] Of these, 4 reported no significant changes,[11,33,35,42] and a significant increase was observed in the other study.[39] Specifically, Rangel-Huerta et al[39] observed significant increases of 0.30 mmol/L and 0.20 mmol/L in the glucose levels of overweight and obese subjects after the consumption of 237 mg/day and 582.50 mg/day of hesperidin in OJ, respectively, for 12 weeks, compared with basal levels. Significant differences were observed between both intervention groups.

Four studies evaluated the effect of hesperidin consumption on plasma insulin levels[11,33,39,42]: 3 of these studies reported no significant changes,[11,33,42] whereas a significant decrease was detected in the other study.[39] Rangel-Huerta et al[39] noted a significant decrease of 1.20 μU/mL in the insulin levels of overweight or obese subjects after the consumption of 237 mg/day of hesperidin in OJ for 12 weeks, compared with basal levels. Significant differences were found between both intervention groups.

The effect of hesperidin consumption on the QUICKI index was evaluated in 2 studies; neither of these studies reported any significant changes.[11,33]

Results for lipid profile parameters. The characteristics of the long-term RCTs included in this review in relation to lipid profiles are detailed in Table 5.

The effect of hesperidin consumption on TC levels was evaluated in 8 studies.[11,33–35,39–42] Of these, 6 reported no significant changes[11,33,34,39,42] and 2 studies reported significant decreases.[35,36] Aptekmann and Cesar[40] found that the TC levels of overweight subjects were significantly decreased by 0.22 mmol/L, compared with basal levels after 13 weeks of consumption of 54.60 mg/day of hesperetin in OJ. No significant differences were observed between the intervention group and the control group. Cesar et al[41] reported a significant decrease of 0.46 mmol/L in the TC levels of hypercholesterolemic subjects who consumed 42 mg/day of hesperetin in 750 mL/day of OJ for 8 weeks, compared with the control subjects. No significant differences were observed between the intervention and control groups.

Eight studies evaluated the effect of hesperidin consumption on LDL-c levels.[11,33–35,39–42] Of these, 6 reported no significant changes,[11,33–35,39,42] while significant decreases were found in the other 2 studies.[40,41] Specifically, compared with the basal level, Aptekmann and Cesar[40] observed a significant decrease of 0.44 mmol/L in the LDL-c levels of overweight subjects after 13 weeks of the consumption of 54.60 mg/day of hesperetin in OJ. No significant differences were observed between the intervention and control groups. Cesar et al[41] observed a significant decrease of 0.49 mmol/L in the LDL-c levels of hypercholesterolemic subjects who consumed 42 mg/day of hesperetin in OJ 8.5 weeks. No significant differences were observed between the intervention and control groups.

The effect of hesperidin consumption on HDL-c levels was evaluated in 8 studies.[11,33–35,39–42] No significant changes were detected in 7 of these studies,[11,33–35,39,41,42] and the other study reported a significant increase.[40] In overweight subjects, Aptekmann and Cesar[40] found that the consumption of 54.60 mg/day of hesperetin in OJ for 13 weeks increased HDL-c levels by 0.23 mmol/L, compared with basal levels. No significant differences were observed between the intervention and control groups.

Table 5 **Characteristics of long-term randomized clinical trials included in the systematic review with results for lipid profiles (n = 8)**

Author, year, reference	Sample size	Cardiovascular risk factors	Nutritional intervention				Results					
			Groups	Flavanone dose	Product dose	Duration	Lipid profile					
							TC, mmol/L	LDL-c, mmol/L	HDL-c, mmol/L	TG, mmol/L	APO A, mg/dL	APO B, mg/dL
Salden et al (2016)[33]	n=6	Overweight or obesity	Placebo capsule vs Hesperidin capsule	0 mg/d 450 mg/d	500 mg/d 500 mg/d	6 wk	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	–	–
Constans et al (2015)[35]	n=25	Hypercholesterolemia	Control drink vs Orange juice (hesperidin)	0 mg/d 213 mg/d	600 mL/d 600 mL/d	4 wk	↓0.35/NS (NS)	↓0.32/NS (NS)	NS/NS (NS)	NS/NS (NS)	NS/↑5.00 (NS)	NS/↑8.00 (NS)
Rangel-Huerta et al (2015)[39]	n=100	Overweight or obesity	Orange juice (hesperidin) vs Enriched orange juice (hesperidin)	237 mg/d 582.5 mg/d	500 mL/d 500 mL/d	12 wk	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	↓0.09/NS (NS)	NS/↓4.00 (NS)	↓4.00/NS (NS)
Rizza et al (2011)[11]	n=24	Metabolic syndrome	Placebo capsule vs Hesperidin capsule	0 mg/d 500 mg/d	1 capsule/d 1 capsule/d	3 wk	NS/NS ($P < 0.05$)*	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)
Morand et al (2011)[42]	n=24	Overweight	Placebo capsule with control drink vs Hesperidin capsule with control drink vs Orange juice (hesperidin)	0 mg/d 292 mg/d 292 mg/d	1 capsule and 500 mL/d 1 capsule and 500 mL/d 500 mL/d	4 wk	NS/NS/NS (NS)	NS/NS/NS (NS)	NS/NS/NS (NS)	NS/NS/NS (NS)	–	–
Demonty et al (2010)[34]	n=194	Overweight Hypercholesterolemia	Placebo capsule vs Hesperidin capsule	0 mg/d 800 mg/d	4 capsules/d 4 capsules/d	4 wk	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	NS/NS (NS)	–	–
Aptekmann and Cesar (2010)[41]	n=26	Overweight	No intervention vs Orange juice (hesperetin)	0 mg/d 54.6 mg/d	0 mL/d 500 mL/d	13 wk	NS/↓0.22 (NS)	NS/↓0.44 (NS)	NS/↓0.23 (NS)	NS/NS (NS)	–	–
Cesar et al (2010)[41]	n=22	Hypercholesterolemia	No intervention Vs Orange juice (hesperetin)	0 mg/d 42 mg/d	0 mL/d 750 mL/d	8.5 wk	NS/↓0.24 (NS)	NS/↓0.49 (NS)	NS/NS (NS)	↑0.38/NS (NS)	–	–

-, parameter not evaluated; ↓, significant decrease between basal and final value within each group; ↑, significant increase between basal and final value within each group; *, significant differences between groups.

Abbreviations: APO A, apolipoprotein A; APO B, apolipoprotein B; HDL-c, high density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; NS, no significant differences between basal and final within each group; (NS), no significant differences between groups; TC, total cholesterol; TG, triglycerides.

Table 6 **Characteristics of long-term randomized clinical trials included in the systematic review with results for coagulation, inflammation, and oxidation biomarkers (n = 6)**

Author, year, reference	Sample size	Cardiovascular risk factors	Nutritional intervention				Results									
			Groups	Flavanone dose	Product dose	Duration	Coagulation biomarkers		Inflammation biomarkers					Oxidation biomarkers		
							FIB	HOM	IL-6, pg/mL	sVCAM-1	sICAM-1	SAA PROTEIN	sE-SEL	sP-SEL	oxLDL, pg/mL	NO
Salden et al (2016)[33]	n=6	Overweight or obesity	Placebo capsule	0 mg/d	500 mg/d	6 wk	–	–	–	NS/NS (NS)	NS/NS (NS)	–	NS/NS (NS)	NS/NS (NS)	–	–
			vs													
			Hesperidin capsule	450 mg/d	500 mg/d											
Constans et al (2015)[35]	n=25	Hypercholesterolemia	Control drink	0 mg/d	600 mL/d	4 wk	NS/NS (NS)	NS/NS (NS)	–	NS/NS(NS)	NS/NS(NS)	–	NS/NS(NS)	–	–	–
			vs													
			Orange juice (hesperidin)	213 mg/d	600 mL/d											
Rangel-Huerta et al (2015)[39]	n=100	Overweight or obesity	Orange juice (hesperidin)	237 mg/d	500 mL/d	12 wk	–	–	–	–	–	–	–	–	NS/NS (NS)	–
			vs													
			Enriched orange juice (hesperidin)	582.5 mg/d	500 mL/d											
Buscemi et al (2012)[36]	n=31	Metabolic syndrome	Control drink	0 mg/d	500 mL/d	1.5 wk	–	–	NS/↓3.30 (P <0.05)*	–	–	–	–	–	–	NS/NS (NS)
			vs													
			Orange juice (hesperidin)	159.5 mg/d	500 mL/d											
Rizza et al (2011)[11]	n=24	Metabolic syndrome	Placebo capsule	0 mg/d	1 capsule/d	3 wk	NS/NS (NS)	NS/NS (NS)	–	NS/NS (NS)	NS/NS (NS)	NS/NS (P <0.05)*	NS/NS (NS)	–	–	–
			vs													
			Hesperidin capsule	500 mg/d	1 capsule/d											
Morand et al (2011)[42]	n=24	Overweight	Placebo capsule with control drink	0 mg/d	1 capsule and 500 mL/d	4 wk	–	–	NS/NS/NS (NS)	NS/NS/NS (NS)	NS/NS/NS (NS)	–	–	–	–	NS/NS/NS (NS)
			vs													
			Hesperidin capsule with control drink	292 mg/d	1 capsule and 500 mL/d											
			vs													
			Orange juice (hesperidin)	292 mg/d	500 mL/d											

-, parameter not evaluated; ↓, significant decrease between basal and final value within each group; *, significant differences between groups.

Abbreviations: FIB, fibrinogen; HOM, homocysteine; IL-6, interleukin-6; NO, nitric oxide; NS, no significant differences between basal and final within each group; (NS), no significant differences between groups; oxLDL, oxidized low-density lipoprotein; SAA protein, serum amyloid A protein; sE-SEL, soluble E-selectin; sICAM-1, soluble intercellular adhesion molecule-1; sP-SEL, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1.

Eight studies evaluated the effect of hesperidin consumption on TG levels.[11,33–35,39–42] Of these, 7 reported no significant changes[11,33–35,39,40,42] and the other study reported a significant decrease.[39] Compared with basal levels, Rangel-Huerta et al[39] observed a significant decrease of 0.09 mg/dL in the TG levels of overweight and obese subjects who consumed 237 mg/day of hesperidin in OJ for 12 weeks. No significant differences were observed between the intervention and control groups.

The effects of hesperidin consumption on apolipoprotein A-1 (Apo A-1) and apolipoprotein B (Apo B) were evaluated in 3 studies,[11,35,39] and different results were obtained. Specifically, compared with basal levels, Constans et al[35] reported a significant increase in Apo A-1 and Apo B levels of 5 mg/dL and 8 mg/dL, respectively, in hypercholesterolemic subjects after the consumption of 213 mg/day of hesperidin in OJ for 4 weeks. No significant differences between the intervention and control groups were observed. Rangel-Huerta et al[39] noted a significant decrease of 4 mg/dL in the Apo A-1 levels and also in the Apo B levels of overweight or obese subjects who consumed 237 mg/day and 582.50 mg/day of hesperidin in OJ for 12 weeks, compared with basal levels. No significant differences were observed between the intervention and control groups. In addition, Rizza et al[3311] found no significant changes between these two groups.

Results for coagulation, inflammation, and oxidative biomarkers. The characteristics of the long-term RCTs included this review in relation to the biomarkers of coagulation, inflammation, and oxidation are detailed in Table 6.

The effect of hesperidin consumption on coagulation biomarkers, assessed based on the plasma levels of fibrinogen and homocysteine, was explored in 2 studies,[11,35] but neither of these RCTs reported any significant changes.

In one study, the effect of hesperidin consumption on inflammation biomarkers was assessed according to plasma protein serum amyloid A (SAA) levels,[11] but no significant changes were observed. Inflammation was also assessed according to plasma IL-6 levels in 2 studies.[36,42] Of these, 1 study observed a significant decrease,[36] but no significant changes were detected in the other study.[42] Buscemi et al[36] found a significant decrease of 3.30 pg/mL in the IL-6 levels of subjects with metabolic syndrome after the consumption of 159.50 mg/day of hesperidin in OJ for 1.5 weeks, compared with basal levels. Significant differences were observed between the intervention and control groups. Four studies evaluated the effects of hesperidin consumption on the plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (s-ICAM-1),[11,33,35,42] and the plasma levels of sE-selectin (soluble E-selectin) and sP-selectin (soluble P-slectin) were evaluated in 3 studies[11,33,35] and 1 study,[33] respectively. None of these studies detected any significant changes.

The effect of hesperidin consumption on oxidative biomarkers was assessed according to plasma NOx levels in 2 studies,[36,42] but no significant changes were observed. Additionally, plasma oxidized low-density lipoprotein levels were assessed in 1 study, but no significant changes were detected.[39]

Effects of acute hesperidin consumption on cardiovascular risk biomarkers. The effects of acute consumption of hesperidin were evaluated according to vascular parameters (SBP, DBP, and endothelial function) and inflammation biomarkers (sVCAM-1, s-ICAM-1, sE-selectin, and sP-selectin) in 2 studies, but no significant changes in any of the investigated parameters were detected.

DISCUSSION

The current systematic review presents a summary of the available scientific evidence regarding the effects of hesperidin consumption on cardiovascular risk biomarkers obtained from animal studies and human RCTs.

The results from the animal studies included in the present systematic review showed that daily consumption of 50–200 mg/kg of body weight of hesperidin or hesperetin for a period ranging from 15 days to 24 weeks significantly lowered blood glucose levels in type 2 diabetic rats and mice. As possible mechanisms of action, other experimental studies with rats have suggested that hesperidin consumption may increase hepatic glycolysis and hepatic glucokinase activity and decrease hepatic gluconeogenesis and hepatic glucose-6-phospatase activity,[43] which would inhibit the gluconeogenic pathway in liver cells[44] and thus prevent the progression of hyperglycemia.[43,45] These beneficial effects on glucose and insulin levels were not observed in the human RCTs included in this systematic review. However, it is interesting to note that only 5 of the 11 RCTs included in the review assessed the effects of hesperidin consumption on blood glucose levels, and the population investigated in these RCTs were overweight, obese, or hypercholesterolemic, whereas the animal studies were performed on type 2 diabetic rats. Because the types of population investigated in the RCTs that evaluated glucose levels yielded no significant results and because only a few RCTs evaluated the possible effect of hesperidin on glucose, more RCTs should be

conducted with type 2 diabetic subjects to assess the effects of hesperidin consumption on glucose and insulin levels in order to confirm the results observed in animals. With respect to insulin levels, no relevant changes were observed in either the animal studies or the human RCTs.

The animal studies included in the present systematic review demonstrated that daily consumption of hesperidin or hesperetin at a dose of 50–200 mg/kg of body weight and 1% or 4.60% of total calorie intake improves the lipid profile by significantly reducing blood levels of TC, LDL-c, and TG in rats and mice with type 2 diabetes and myocardial ischemia. An in-vitro study showed that the possible mechanism through which hesperidin improves the lipid profile may involve the modulation of hepatic lipid metabolism and the inhibition of Apo B in HepG2.[46] In contrast, the results of the RCTs included in this review did not show the same conclusive results. In fact, only 2[40,41] of the 8 articles that assessed lipid profiles observed a decrease in TC and LDL-c levels. Interestingly, only one study[41] assessed the effect of hesperidin on lipid profile in hypercholesterolemic subjects. This RCT observed marked decreases of 0.47 mmol/L and 0.49 mmol/L in TC and LDL-c levels, respectively,[47] after the consumption of 42 mg/day of hesperidin in OJ for 8.5 weeks, and this finding was clinically relevant.[47] Thus, hypercholesterolemic subjects constitute an appropriate population for further evaluation of the specific effects of hesperidin on lipid profile. The differences between the doses of hesperidin administered in the animal and human studies (higher doses were used in the animal studies than in the human RCTs) may also have contributed to the difference in the results obtained from these two types of studies. Thus, more human RCTs are needed to better understand the effects of hesperidin consumption on lipid profile in humans.

The present systematic review showed that, in animal models, the consumption of hesperidin does not improve anthropometric parameters, such as body weight and visceral fat. However, it is important to note that the animal studies included in this review were conducted with rats or mice with normal body weight and anthropometric parameters for their age; future studies should investigate overweight or obese rats or mice to allow more relevant conclusions to be drawn. Similarly, in the human RCTs, there were no effects of hesperidin on body weight, BMI, and body fat, and only a limited number of studies have assessed these parameters. Two[39,40] of the 3 RCTs that evaluated the effect of hesperidin consumption on body weight and BMI observed reductions of 1.30–1.80 kg/m^2 and 0.30–0.70 kg/m^2, respectively, in overweight subjects after daily consumption of 54.60–582.50 mg/day of hesperidin in OJ for 12–13 weeks, compared with the basal values. However, both of these studies had some limitations: one was not a placebo-controlled clinical trial,[39] and the other study observed decreases in both the intervention and control groups,[40] probably owing to the fact that volunteers tend to pay more attention to their health when participating in a study.[48]

Hesperidin has aroused interest on account of its possible effect on blood pressure because it has been suggested that this compound exerts effects similar to those found with other flavonoids, such as quercetin.[49] In-vitro studies have shown that the improvements in blood pressure and endothelial dysfunction observed after hesperidin consumption may be mediated by a decrease in NADPH oxidase 2, increase in plasma NO metabolites, and an inhibitory effect on angiotensin-converting enzyme.[50,51] These data suggest that hesperidin may increase the secretion of NO by human endothelial cells, inhibit cyclic nucleotide phosphodiesterase, and increase cyclic AMP (adenosine monophosphate) and GMP (guanosine monophosphate), thereby exerting a vasorelaxant effect.[14,52,53] Nevertheless, according to the findings of the present review of animal studies and RCTs, the consumption of hesperidin has no clear effect on DBP and SBP levels. However, it is interesting to note that the subjects assessed in the included RCTs were overweight or obese, with no hypertension or elevated blood pressure levels. Therefore, studies that evaluate the effect of hesperidin on blood pressure in subjects with high blood pressure levels are needed for us to draw a definitive conclusion about this CVRF. Interestingly, 3 RCTs [11,33,36] included in the present review assessed the effects of hesperidin on endothelial function, and 2 of these[11,36] observed improvements in these parameters in subjects with metabolic syndrome and increased CVRFs after 1.5–3 weeks of intervention with 300–500 mg/day of hesperidin in OJ or capsule form. Although the available evidence is scarce, it appears that hesperidin consumption seems likely to increase endothelial function. Thus, more human RCTs are needed to determine whether hesperidin decreases blood pressure and improves endothelial function in hypertensive or type 2 diabetic populations.

The results obtained in the present review of RCTs showed that hesperidin has no significant effects on biomarkers of coagulation, inflammation, and oxidation. However, few studies have assessed the effect of hesperidin on these biomarkers in relation to CVDs because almost all studies have focused on cancer and other chronic diseases.[26,54,55]

One factor to consider is the interindividual variability in hesperidin bioavailability, which may, for example, depend on the microbiota composition of each subject.[56,57] Thus, it is possible that different individuals

administered the same dose of hesperidin can absorb this compound to different degrees, and therefore, these individuals would show different effects for the various cardiovascular biomarkers. This could also explain the differing results between the studies included in this review because none of the studies considered the bioavailability of hesperidin.

The RCTs included in the present review that observed more significant changes[39–41] presented many potential risks of bias, which were classified as unclear risk owing to insufficient information about allocation concealment and blinding of participants, personnel, and outcomes, or in terms of including a conflict of interest based on the Cochrane risk of bias criteria. These unclear risks of bias indicate potential problems related to the methodological quality of the studies and hence lead us to question the reliability of the results of the RCTs. Therefore, further RCTs are needed with a lower risk of bias and consequent improvement in quality.

One strength of this review concerns the standardized methodology that was used. In addition, the included studies were published recently and thus presented strong scientific evidence, such as RCTs, along with analyses of their individual risks of bias. Moreover, the novelty of this review lies in the fact that it was the first to evaluate the effects of hesperidin consumption on different CVRFs based on both animal models and human studies. However, the present review has several limitations that warrant discussion. The first is the scarce scientific evidence available from human and animal studies that assessed the effects of hesperidin on CVRFs. In most studies, the populations used to evaluate the effects of hesperidin on different CVRFs have not been the most appropriate for reaching definitive conclusions. Thus, if the objective of a study is to improve a specific cardiovascular risk factor – for example, to reduce high serum cholesterol concentrations in humans – the recommendation is to include subjects that present with symptoms associated with this specific CVRF, such as hypercholesterolemic patients.[58] In addition, the studies included in this review utilized different intervention durations, monitoring approaches, and methods of supplementation. However, the sample size in some of the animal studies was perhaps insufficient for a robust evaluation of the objectives, and in 2 studies, the doses of hesperidin or hesperetin were not estimated in milligrams, and therefore their dose-dependent effects could not be compared with those of other studies. In addition, dose- and time-dependent effects, as well as the physiological relevance of the dose used, were not evaluated in the animal studies. Also, the possibility of residual confounding related to hesperidin bioavailability cannot be excluded. Moreover, even though compliance with the nutritional intervention is necessary, dietary factors may not have been considered to a sufficient degree because only 3 RCTs controlled the participants' diet through validated dietary records, and no biomarkers for consumption were used in any of the included studies. Therefore, other polyphenol compounds present in the diet may have been responsible, either partially or entirely, for the observed health effects. In addition, with inadequate monitoring of the participants' diet, it is possible that some subjects had greater hesperidin intake than others because they consumed food or beverages with significant amounts of hesperidin, potentially affecting the study results of the study. Thus, in nutritional RCTs, monitoring of the participants' diet is necessary to avoid confounding between other dietary compounds and the dietary intervention. Limiting hesperidin intake as a dietary recommendation for all participants, monitoring their dietary intake, and the use of biomarkers for consumption are necessary to obtain robust results in this type of study. Lastly, most of the articles included in this review lacked statistical data, such as mean differences and their standard deviation and the standard error or confidence intervals for each intervention, as well as their p-values. Consequently, a meta-analysis, which would have provided more conclusive results, as well as a forest plot, which would have provided a clearer presentation of the results, could not be performed.

CONCLUSION

In conclusion, hesperidin consumption was found to improve glucose levels and various lipid profile parameters, such as TC, LDL-c, and TG, in animal models, but no definitive conclusion regarding the effects of hesperidin on different CVRFs in humans can be currently drawn. Further RCTs of greater quality are needed to confirm that the results observed in animal models can be translated to the human population and thus to evaluate whether the administration of hesperidin through the consumption of citrus food or as a supplement would serve as a new tool for the prevention and treatment of CVDs.

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Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **Characteristics and results of animal studies included in the systematic review (n = 12)**

Table S2 **Characteristics and results of randomized clinical trials included in the systematic review (n = 11)**

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