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Article

Analysis of Beneficial Effects of Flavonoids in Patients with Atherosclerosis Risk on Blood Pressure or Cholesterol during Random Controlled Trials: A Systematic Review and Meta-Analysis

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Abstract: Flavonoids are plant-secondary metabolites with cardiovascular protective properties. Few studies have examined specific flavonoid classes or pure flavonoids concerning some common cardiovascular risks. To obtain information in a systematic review to analyze in a meta-analysis, data were recovered regarding flavonoid intake in random controlled trials and atherosclerosis disease, related to risk factors such as blood pressure, total cholesterol (TC), and low-density lipoprotein cholesterol (LDLc). Our aim was to conduct a meta-analysis using the Scopus and PubMed databases without restrictions on the year of publication, extracting articles over the period 1–15 April 2023, searching for randomized controlled trials (RCTs) that investigated different types of flavonoids, measuring blood pressure and low-density cholesterol plasmatic concentration. This paper's Prospero registration is CRD 42023414153. There were 19 RCTs: twelve RCTs were considered for blood pressure data analysis and fifteen RCTs for total cholesterol and LDL cholesterol data analysis. The meta-analysis showed no significant differences between placebo treatments and treatments with different flavonoids on blood pressure. However, there was a significant difference found in quantitative analysis for TC and LDLc. In conclusion, flavonoid consumption can be associated with a lower risk of LDLc and TC, and more RCTs are needed to specify the effect of more types of pure flavonoids in atherosclerotic patients.

Keywords: flavonoids; atherosclerosis; cholesterol; LDL; blood pressure; systematic review; meta-analysis

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1. Introduction

Atherosclerosis is the main underlying cause of cardiovascular diseases, and these are the leading cause of death globally. Atherosclerosis is also the leading cause of death in low- to middle-income countries [1]. Some risk factors contributing to the development of cardiovascular diseases and atherosclerosis are hypertension, dyslipidemia, diabetes mellitus, visceral obesity, and smoking [2]. These factors induce the expression of cytokines,

including TNF-alpha, IL1-alpha, IL1-beta, IL-6, macrophage colony-stimulating factor (M-CSF), IL-18, and vascular adhesion molecule-1 (VCAM-1) by endothelial cells. All of these contribute to the progress of atherosclerosis by recruiting monocytes to the intima vascular layer [3]; fortunately, the inflammation-mediated damage is reversible. Pharmacologic interventions aiming to reduce the morbidity and mortality of cardiovascular diseases include (usually as first-line treatments) statins, beta blockers, angiotensin converting enzyme (ACE) inhibitors, and antiplatelets drugs, but these generally present adverse side effects, such as headaches, muscle pain, sleep problems, bleedings, hypotension, and fatigue, among many others [4,5]. In the first stages of atherosclerosis, conservative measures, such as lifestyle changes, are an option to control some of the risk factors and avoid cardiovascular disease development [5,6]. These lifestyle changes may include the supplementation of phytochemicals that have proven beneficial effects on some biochemical parameters. Some natural compounds have shown beneficial effects on blood pressure parameters and plasma lipid concentrations, two common risk factors for atherosclerosis. One of the most studied natural compound groups is flavonoids, which represent the most extensive family of polyphenolic compounds with antioxidant properties and are present in a wide variety of foods [5,7]. Flavonoids can be subdivided into different groups depending on the substitution pattern: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins [8]. Their presence in plants and foods varies widely, not only in terms of concentration but also in terms of flavonoid content or flavonoid combination. Some plants contain only one flavonoid, such as pandan (*Pandanus tectorius*), which contains only tangeretin, a flavone that is associated with helping to lower plasmatic cholesterol concentrations [9]. On the other hand, other plants produce many flavonoids from the same group; for example, Mongolian milkvetch (*Astragalus membranaceus*) contains flavones, which prevent atheroma plaque formation [10]. Regarding foods, onion contains only quercetin, which is known for its anti-inflammatory, antioxidant, and hypolipidemic effects. Meanwhile, wine grapes contain anthocyanins, flavonols, flavanols, dihydroflavonols, and proanthocyanidins [11], which are known to protect against cardiovascular diseases and inflammatory states. Thus, even though many studies have elucidated the individual biochemical effects of different flavonoids in vitro, in terms of antioxidant power, the inhibition of specific inflammatory cytokines, the blocking of foam cell formation during atherosclerosis, and so on, clinical effects and total benefits, based on the concentration and class of flavonoid, still need to be clarified, in order to establish clear and reliable recommendations for each cardiovascular pathology.

The dietary consumption of flavonoids is related to decreased morbidity and mortality in cardiovascular diseases. Their effect on the clinical progression of diseases depends on their antioxidant activity [12]. The most consumed flavonoids are flavanols, because they are the most abundant group in fruits and vegetables. Flavones are common in our diet too, but in lesser quantities [13].

Rather than total flavonoid intake effects, the benefits of specific doses and classes of flavonoids should be analyzed to find a better use of these compounds in human healthcare [14]. Therefore, it is necessary to demonstrate, via randomized controlled trials, that some dietary interventions can help to prevent atherosclerosis and, consequently, many other cardiovascular diseases [15]. Meanwhile, the previously performed clinical trials have used flavonoids or classes of flavonoids to address the parameters involved in the development of atherosclerosis in different populations who are at risk of cardiovascular disease, such as post-menopausal women and individuals with obesity and dyslipidemia. It is important to address the current knowledge of the real benefits of different flavonoids in the pathophysiology of atherosclerosis. There were four meta-analyses performed in order to analyze the benefits of flavonoids in general during cardiovascular diseases: one in 2008 [16]; one in 2015 [17]; one in 2017 [18], which analyzed mortality risk specifically; and another in 2021 [19], addressing the dose–response benefits for coronary heart disease (CHD). To our knowledge, this is the first meta-analysis addressing the effects of pure flavonoids on the development of atherosclerosis. The advantage of analyzing early stages

of CVD is the fact that conclusions regarding the specific classes and doses of flavonoids can help to improve lipid and blood pressure parameters as a prevention for developing the disease. Interventions in early stages not only inhibit severe events or mortality, but also really improve the health status of patients with significant changes in serum biomarkers.

A biomarker is an indicator of pharmacological responses, normal biological, or pathogenic processes during a therapeutic intervention [20]. For example, hypercholesterolemia is an early biomarker of atherosclerosis [21]. Other risk factors and biomarkers are systolic and diastolic blood pressure higher than 130- and 80-mm Hg, respectively; total cholesterol concentration above 199 mg/dL or 5.15 mmol/L; and LDLc higher than 129 mg/dL, or 3.3 mmol/L [20]. These biomarkers were chosen because they have a big prevalence in our country.

In this review, we present the results of two meta-analysis performed to the results reported in 19 randomized controlled trials regarding the effect of the consumption of a specific type or class of flavonoid on blood pressure and cholesterol or LDLc concentrations.

2. Materials and Methods

This systematic review was prepared according to the Preferred Reporting Items of Systematic Reviews and Meta-Analyses [22]. Searches of the literature were conducted without restrictions of publication year in order to find studies that only utilized a single class of flavonoid in association with total cholesterol concentrations or low-density cholesterol concentrations and/or blood pressure. The search was performed using two databases, Scopus and PubMed, to obtain articles between 1 April 2023 and 15 April 2023. Searches were filtered to find words directly in the search box without any NCBI filters or any date restrictions. In Scopus, terms were used directly in the search box using the filter "Article title, Abstract, Keywords". For each database, the terms used were: [flavonoids OR anthocyanin OR benzoflavone OR biflavonoid OR catechin OR chalcone OR flavone OR flavonolignans OR flavonol OR isoflavone OR phloretin OR proanthocyanidins] and [atherosclerosis OR peripheral arterial disease] and [cholesterol OR LDL] and [blood pressure OR arterial pressure]. All terms belong to the medical subject headings (MESH). Thirty-one documents were obtained in Scopus while in PubMed, we retrieved forty-eight documents. Three duplicated publications and an article in a different language other than English were removed. 15 April 2023 was the last date of research. The flow diagram of the search and selection is shown in Figure 1.

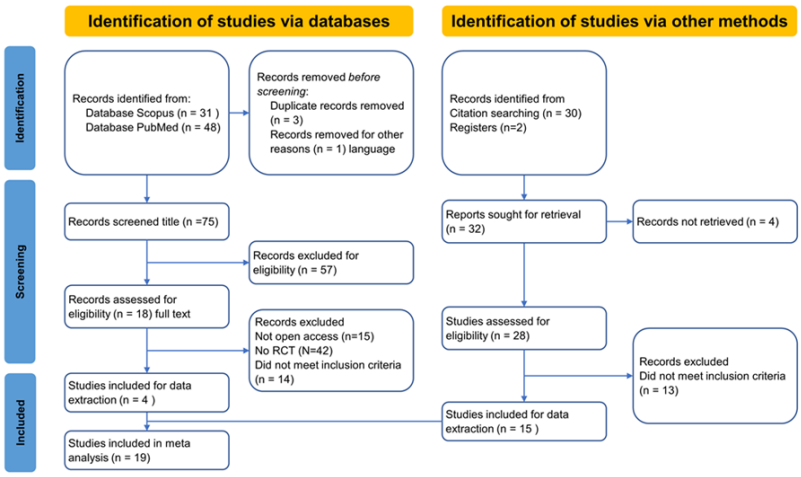

Figure 1. Flow diagram of the literature search as a systematic review and meta-analysis (based on Page et al. template) [22].

Subsequently, two reviewers assessed the resulting publication titles and abstracts that were retrieved from the aforementioned search. The articles were screened for relevance and all potentially eligible abstracts were evaluated in full text. Studies that did not report

results from a specific type or class of flavonoid were excluded. Books, letters, editorials, and case reports were also excluded. Only RCT studies using purified flavonoids, or a single class of flavonoid content were considered for meta-analysis. Through evaluation of the referenced bibliography of the identified articles, we retrieved a total of thirty new studies and two registrations, from which fifteen fulfilled criteria to be added to the final count.

2.1. Data Extraction and Quality Assessment Tool

Two reviewers assessed full-text articles and extracted useful information to record in a Microsoft Excel spreadsheet. Mean and standard deviation of TC, LDLc, systolic blood pressure (SBP), diastolic blood pressure (DBP), and number of participants in treated and control groups were included in the analysis. Additional information such as sex, age, treatment duration, flavonoid dose, type of RCT analysis, and participant health conditions at the beginning of the intervention were included in Table 1.

Table 1. Intervention characteristics from each study.

Author	Flavonoid or Class of Flavonoid	Participant		Treatment		RCT Analysis Style	Participants Characteristics
		Sex	Age	Length	Dose mg/d		
Samman et al., 1999 [23]	Isoflavones	F	18–45	4 months	86	Single blind, crossover	Premenopausal women
Wang et al., 2004 [24]	Isoflavones	M, F	Over fifty	6 weeks	92	Double blind, crossover	Hypercholesterolemic subjects
Hodgson, 2005 [25]	Catechins	M, F	45–70	4 weeks	300	Single blind, parallel	History of coronary artery disease
Wang-Polagruto, 2006 [26]	Flavanols	F	53–59	6 weeks	446	Double blind, parallel	Hypercholescterolemic postmenopausal women
Nestel et al., 2007 [27]	Isoflavones	M, F	50–64	5 weeks	1000	Double blind, crossover	Overweight men and postmenopausal women
Widlansky et al., 2007 [28]	Catechin	M, F	48–68	2 weeks	300	Double blind	Men and women with coronary artery disease
Qin et al., 2009 [29]	Anthocyanins	M, F	40–65	12 weeks	320	Double blind, parallel	Dyslipidemic patients
Zhu et al., 2011 [21]	Anthocyanin	M, F	40–65	12 weeks	320	Double blind, parallel	Hypercholesterolemic individuals
Pfeuffer et al., 2013 [30]	Flavonols	M	48–68	8 weeks	150	Double blind, crossover	Men with APOE4 polymorphism
Zhu et al., 2013 [31]	Anthocyanin	M, F	40–65	24 weeks	320	Double blind, parallel	Subjects with hypercholesterolemia
Koutelidakis et al., 2014 [32]	Catechins	M, F	45–70	3 hours	400	Single blind, parallel	Patients with coronary artery disease
Davinelli et al., 2015 [33]	Anthocyanin	M, F	45–65	4 weeks	162	Double blind, parallel	Overweight and smokers
Johnson et al., 2015 [34]	Anthocyanin	F	45–65	8 weeks	103.2	Double blind, parallel	Promenopausal women with pre and stage 1 hypertension
Zhang et al., 2016 [35]	Anthocyanin	M, F	40–65	24 weeks	320	Double blind, parallel	Hypercholesterolemic individuals
Argani et al., 2016 [36]	Proanthocyanidins	M, F	21–64	8 weeks	190	Double blind, parallel	Moderate hyperlipidemia
Nogueira et al., 2016 [37]	Catechins	F	18–59	4 weeks	780	Double blind, crossover	Prehypertensive obese individuals
Salden et al., 2016 [38]	Flavanone	M, F	40–68	6 weeks	450	Double blind, parallel	Overweight individuals
Hollands et al., 2018 [39]	Procyanidin	M, F	56–70	4 weeks	130	Double blind, crossover	Men and Women with moderately high blood pressure
Capomolla et al., 2019 [40]	Flavanones	M, F	40–80	90 days	650, 1300	Double blind	Metabolic syndrome, obese, and mild hyperglycemia

The eligibility and quality criteria were based on: complete information, article content and a single class of flavonoid studied. The evaluation tool was based on six items: (1) human trials; (2) randomized controlled trials; (3) analysis of a single class of flavonoid; (4) existence of a control group; (5) post-treatment SBP and DBP recorded data; and (6) outcome measurement of TC and LDLc. Eligibility criteria consisted of obtaining at least the first four points and one or both of the last two points. Participants were required to be considered population at risk of developing atherosclerosis.

2.2. Outcome Measures

SBP and DBP were assessed at the end of the treatment in twelve RCTs, and TC and LDLc were assessed in fifteen RCTs. A meta-analysis was performed for each outcome.

2.3. Risk of Bias in Individual Studies

One author assessed the risk of bias using a table following the RoB 2.0 Cochrane tool, reporting results for each study (Table 2). The potential sources of heterogeneity and publication bias were explored using the algorithms in the RoB 2.0 Cochrane tool, following the instructions for each section.

2.4. Statistical Analysis

A meta-analysis for each considered dependent variable was performed using the R program version 4.1.2 (1 November 2021) [41]. Since the analysis was based on different flavonoids or working conditions, the random effect model was implemented. The selected effect size was the standardized mean difference with the Hedges' g bias correction for small sample sizes. The inverse variance method was used to obtain a weighted mean; the DerSimonian–Laird method was used to estimate tau2 (between-study variance), and the Jackson method for the confidence interval for tau2 and tau [42].

3. Results

Searches identified a total of seventy-nine articles in Scopus and PubMed databases, from which four articles were removed due to duplication (three) and language criteria (one) (Figure 2). During the screening, fifty-seven studies were excluded from eligibility using the open access filter in Scopus and randomized controlled trial in PubMed. Eighteen records were eligible for assessment as full text from which fourteen did not meet inclusion criteria. Four full texts were included for meta-analysis. Additional fifteen articles could be recovered by other means such as citation searching and registers' review. Citation searching was elaborated as a complement and thirty studies were identified, from which thirteen were excluded because they did not meet inclusion criteria. Four were not retrieved. In addition, two registers from articles were donated to our team. Subsequently, fifteen more articles were included for meta-analysis. Finally, nineteen articles were included in the quantitative analysis.

We performed meta-analyses to show through statistical methods the presence or absence of quantitative differences between studies' outcomes. SBP showed a summary effect with a non-significant Standardized Mean Difference (SMD) of -0.21 ($p = 0.2007$); thus, the flavonoid treatment did not show any effect on this parameter. Furthermore, the heterogeneity ratio was high between studies with an $I^2 = 79\%$, and the differences were statistically significant concerning heterogeneity ($\tau^2 = 0.2503$, $p < 0.01$, Figure 3). The summary effect of DBP had a SMD = -0.18, ($p = 0.2869$) for the meta-analysis result, showing no effect of flavonoids on this parameter. In conclusion, there is no effect of the treatments on blood pressure levels (Figures 2 and 3). The summary effect for TC showed a SMD = -0.30 ($p = 0.0499$). It shows that there is a significant effect of the treatments for TC plasma concentrations (Figure 4). Finally, LDLc had a summary effect of SMD = -0.34 ($p = 0.01$), showing a significant effect of treatments on this plasma parameter, presenting a high heterogeneity between studies ($I^2 = 74\%$), which was statistically significant ($\tau^2 = 0.1865$, $p < 0.01$, Figure 5).

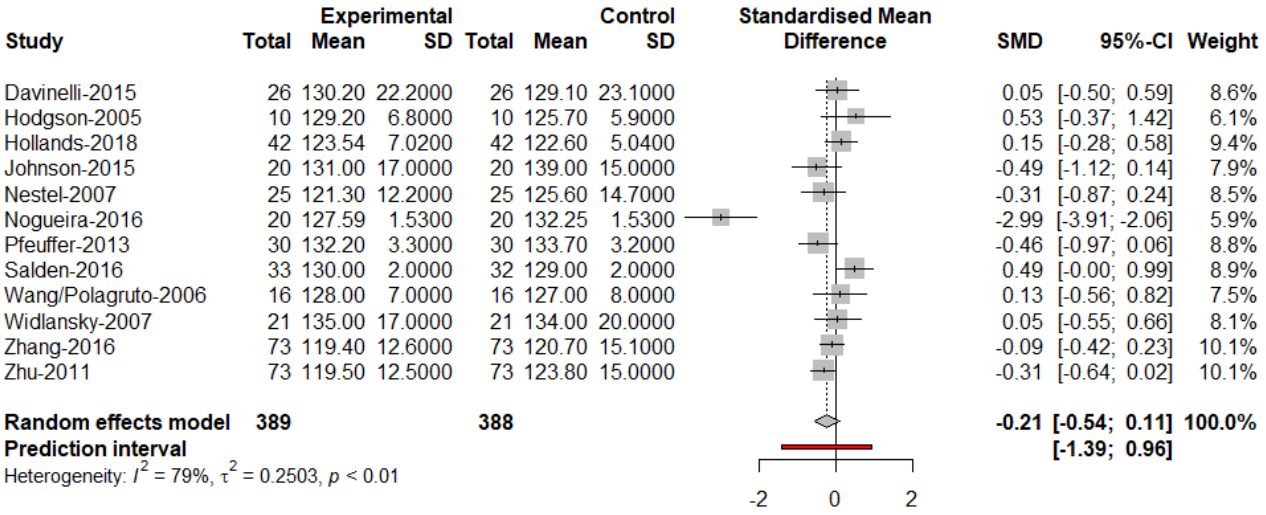

Figure 2. Forest Plot of SBP means. Comparison between two groups: control and experimental group. Squares: Size effect of each study; Diamond: final meta-analysis result; Red line: total confidence interval. The result from meta-analysis has a *p*-value = 0.2007. The control group from each study is compared with the experimental group to get the confidence interval of 95%, squared mean deviation (SMD), and the pondered contribution weight; this corresponds to the size of squares. Included studies for meta-analysis in Refs. [21,23–40].

Table 2. Risk of bias in individual studies. Plus sign indicates low or low risk, and interrogation sign indicates some concerns for the bias.

Risk of Bias / Study	From the Randomization Process	Due to Deviations from Intended Interventions	Due to Missing Outcome Data	In Measurements of the Outcome	In Selection of the Reported Result
Argani et al., 2016 [38]	+	+	+	+	+
Davinelli et al., 2015 [35]	+	+	+	+	+
Hodgson 2005 [27]	+	+	+	+	+
Hollands et al., 2018 [41]	?	+	+	+	+
Johnson et al., 2015 [36]	+	+	+	+	+
Koutelidakis et al., 2014 [34]	+	+	+	+	+
Nestel et al., 2007 [29]	+	+	+	+	+
Nogueira et al., 2016 [39]	+	+	+	+	+
Pfeuffer et al., 2013 [32]	?	?	+	+	+
Qin et al., 2009 [31]	+	+	+	+	+
Salden et al., 2016 [40]	+	+	+	+	+
Samman et al., 1999 [25]	+	+	+	+	+
Capomolla et al., 2019 [42]	?	+	+	+	+
Wang et al., 2004 [26]	+	+	+	+	+
Wang-Polagruto 2006 [28]	+	+	+	+	+
Widlansky et al., 2007 [30]	+	+	+	+	+
Zhang et al., 2016 [37]	+	+	+	+	+
Zhu et al., 2011 [21]	?	+	+	+	+
Zhu et al., 2013 [33]	+	+	+	+	+

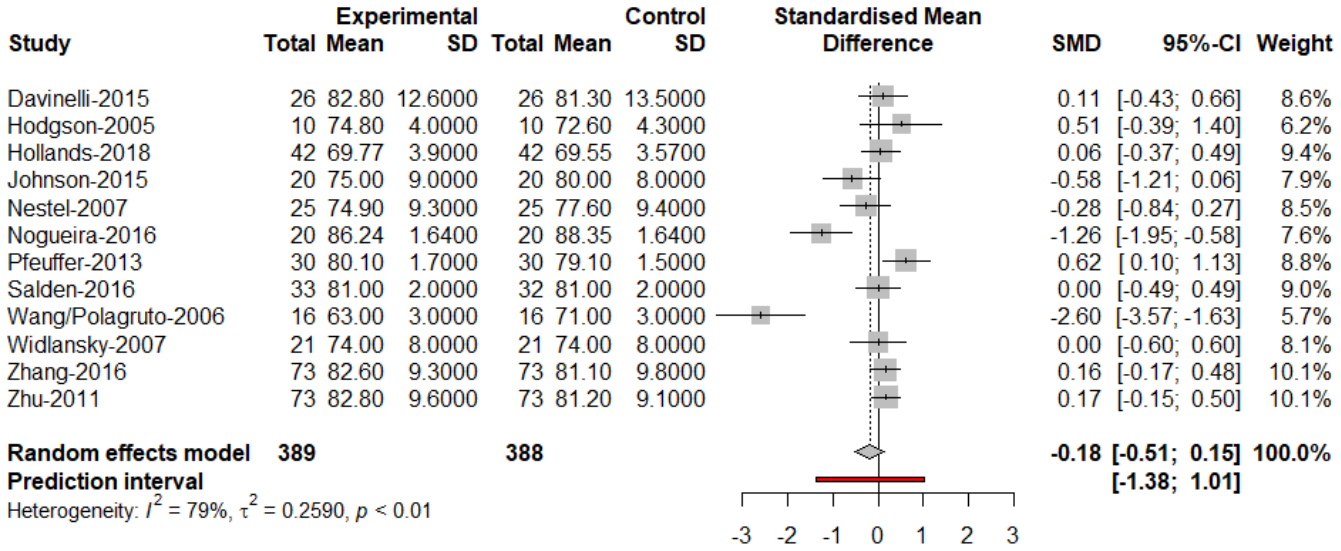

Figure 3. Forest Plot of DBP means. Comparison between two groups: control and experimental group. Squares: Size effect of each study; Diamond: final meta-analysis result; Red line: total confidence interval. The result of this meta-analysis gives us a *p*-value = 0.2869. The control group from each study is compared with the experimental group to get the confidence interval of 95%, squared mean deviation (SMD), and the pondered contribution weight; this corresponds to the size of squares. Included studies for meta-analysis in Refs. [21,23–40].

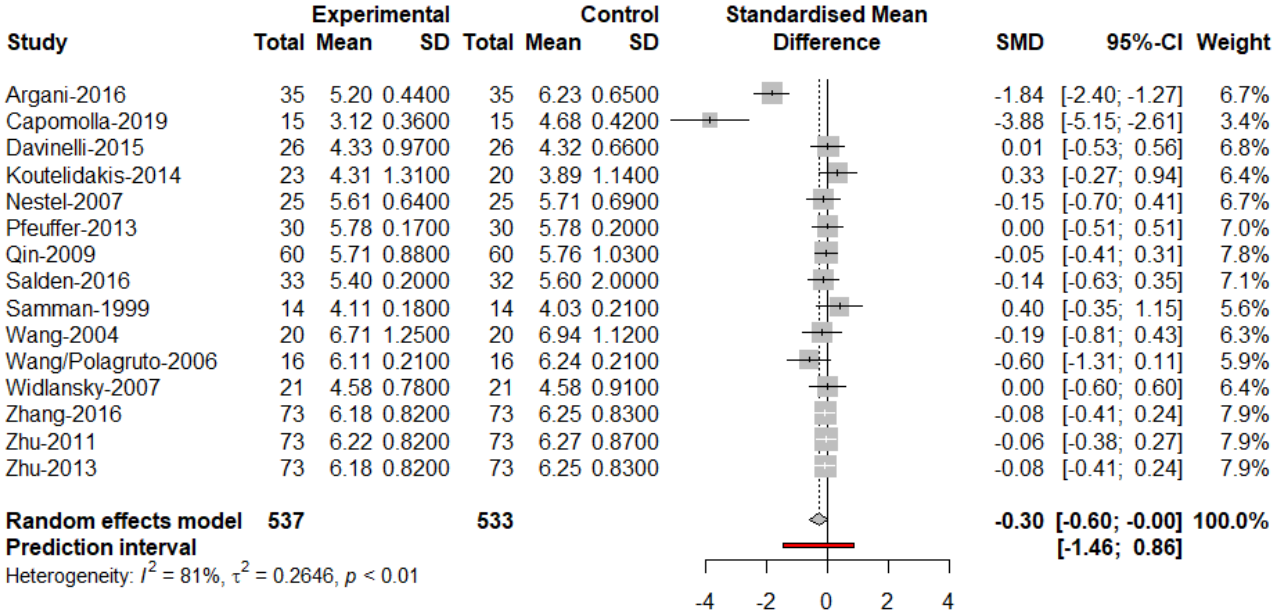

Figure 4. Forest Plot of TC means. Comparison between two groups: control and experimental group. Squares: Size effect of each study; Diamond: final meta-analysis result; Red line: total confidence interval. The result of this meta-analysis gives us a *p*-value = 0.0499. The effect during the use of flavonoids is significative on TC. The control group from each study is compared with the experimental group to get the corresponding confidence interval of 95%, squared mean deviation (SMD), and the pondered contribution weight; this corresponds to the size of squares. Included studies for meta-analysis in Refs. [21,23–40].

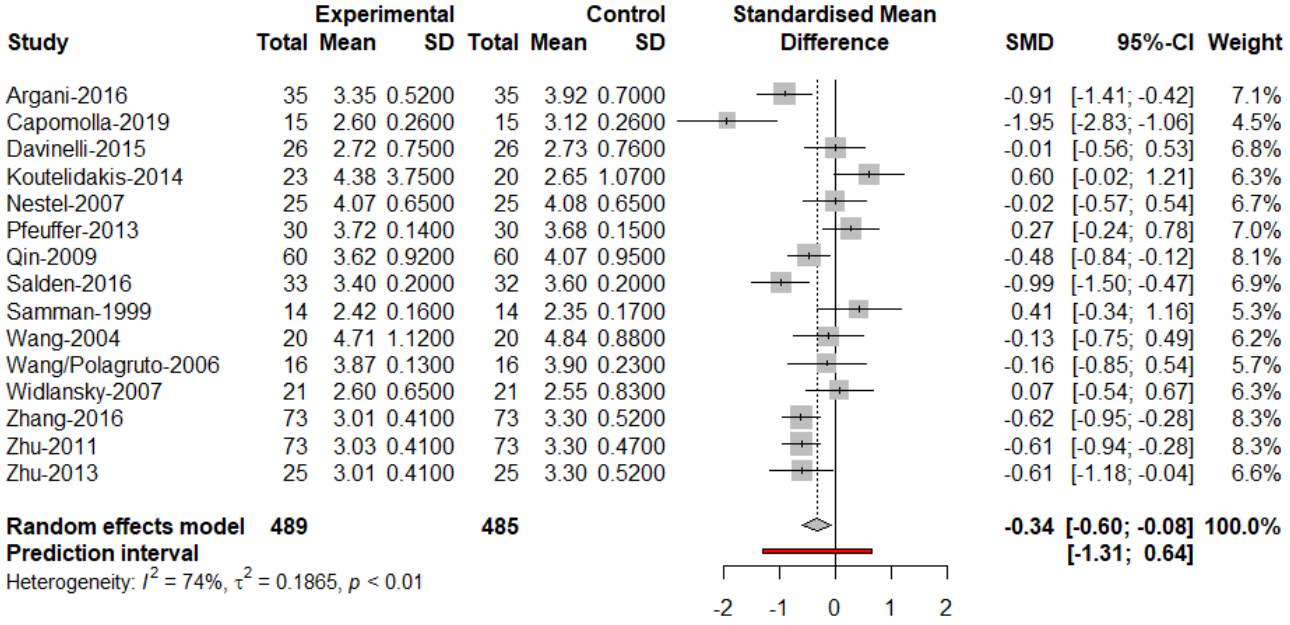

Figure 5. Forest Plot LDLc means. Comparison between two groups: control and experimental group. Squares: Size effect of each study; Diamond: final meta-analysis result; Red line: total confidence interval. The *p*-value = 0.0113, for this meta-analysis. The effect during the use of flavonoids is significative on LDLc. The control group from each study is compared with the experimental group to get the confidence interval of 95%, squared mean deviation (SMD), and the pondered contribution weight; this corresponds to the size of squares. Included studies for meta-analysis in Refs. [21,23–40].

4. Discussion

According to the results of our meta-analyses, the quantitative assessment revealed that flavonoid intake is adequate to achieve lower TC and LDLc concentrations after consumption. However, it does not show benefits on blood pressure parameters. Most studies did not find significant effects on SBP [21,25–28,30,33–35,38,39] (Figure 2) or DBP [21,25,27,28,30,33–35,38,39] (Figure 3) using catechins, flavonols, anthocyanins, flavanones, proanthocyanidins, or isoflavones. However, catechins and anthocyanins had a significant effect in two individual studies [37,38]; the differences are not related to the flavonoid group, (Figures 2 and 3). This negative result could be explained by the difference in flavonoid sources used in each study.

It has already been reported that flavonoids with structural characteristics of anthocyanins have a good lowering effect on LDLc and TC plasma concentrations [29]. Other groups of flavonoids that showed significant effects on LDLc or TC plasma concentrations are flavanones and proanthocyanidins (Figures 4 and 5) [36,40]. The mechanisms of action that could explain the in vivo observed differences are still poorly understood. As antioxidants, flavonoids have different mechanisms related to their structural characteristics and free radicals. When flavonoids present ortho hydroxyl groups in the B ring at position C4′ and C5′, or C3′ and C4′, they exert better antioxidant characteristics, as is the case of the flavonol quercetin. All C3-OH or C5-OH flavones present a tautomeric form that is able to inhibit pro-oxidant enzymes. Hydroxyl radicals or peroxide formation is avoided with both structures. Additionally, two neighbor oxygens can act as metal chelators, preventing hydroxyl radicals or peroxide formation. Some flavonoids can inhibit lipoxygenases because they present a double bound between C2 and C3, a carbonyl group in C4 and one catechol group in B ring. The aglycones are less water-soluble than their respective glycosides, and more reactive to protect lipids [43]. Despite these known mechanisms of antioxidation, many molecular mechanisms during the physiopathology of oxidation-associated diseases need to be clarified in detail in order to understand the specific benefits of each flavonoid at specific disease stages.

During the past years, some meta-analyses focused on analyzing the effect of isoflavones on LDL concentrations. Weggemans et al., in 2003, analyzed soy-associated isoflavones on cholesterol concentrations in 10 clinical trials; the authors did not find changes in LDL concentration after intervention with soy protein. However, it is worth mentioning that the isoflavones' bioavailability was different between the administered soy samples. Similarly, we did not find significative improvement in LDL concentrations after isoflavone consumption, although there was a lowering effect. These similarities could be explained due to the fact that the subjects analyzed in the aforementioned study were patients with hypercholesterolemia. Comparably, our study examined subjects with similar conditions of dyslipidemia, such as obesity, or post-menopausal women [44]. Zhuo et al., in 2004, found in a meta-analysis with only eight studies, that high isoflavone intake led to significantly greater decreases in serum LDLc than low isoflavone intake, with the same quantity of soy protein ingestion, demonstrating that isoflavones have LDLc-lowering effects independent of soy protein. These results support the discussion about bioavailability [45]. In the case of the last study, Taku et al. (2007) found in a meta-analysis of eleven studies that isoflavones significantly decreased LDLc and TC, and the reduction was larger in hypercholesterolemic individuals than in normocholesterolemic subcategory [46].

Regarding a meta-analysis that evaluates the effect of flavonoids on blood pressure, Raman et al. [47] reported in their meta-analysis improvements in patients' lipid profile and systolic and diastolic pressure using flavan-3-ols as treatment. However, they found considerable heterogeneity and recommended future studies with high-quality dose–response assessments. In the case of quercetin, Serban et al. [48], in their systematic review and meta-analysis, showed significant reductions in systolic and diastolic blood pressure. Daneshzad et al. [49] found through a meta-analysis that anthocyanin supplementation had significant effects on TC and LDLc using more than 300 mg/day during more than twelve weeks and had no effects for systolic and diastolic blood pressure similar to our results. In a qualitative analysis, Sone et al. [50] did not find a significant difference with catechin treatment in any of the measured cardiovascular diseases (CVD) risk factors, including TC and LDLc.

To our knowledge, there are four previous meta-analyses in the literature considering flavonoids and their benefit for cardiovascular disease: Hooper et al. in 2008 could be considered the predecessor of this work; they included 133 clinical trials and analyzed different flavonoids and sources, and their effect on cholesterol concentrations, blood pressure, and CVD morbidity and mortality. Most studies included in Hooper and colleagues' meta-analysis used a mix of flavonoids for the corresponding interventions. They found that chocolate improved systolic and diastolic pressures, soy protein isolates improved diastolic blood pressure and LDLc, and green tea reduced LDLc [16]. These results are in good agreement with ours. Hooper and colleagues suggested the analysis of dose–response effects and the analysis of other types of flavonoids, like anthocyanins and flavanones, for future research. A study by Jiang et al. (2015) analyzed the risk of CHD in fifteen prospective studies. Even though no linear dose–response association was found, the intake of higher amounts of flavonoids was associated with a lower risk of CHD in European and American studies. However, no association to a specific class of flavonoid was reported [17]. Liu et al. (2017) analyzed mortality from all causes in CVD in ten prospective studies. The authors found strong evidence for the recommendation of consuming flavonoids-rich food to reduce risks of mortality. However, once again, no specification of flavonoid was reported [18]. Finally, Micek et al. (2021) showed that an increasing dietary intake of total flavonoids is linearly associated with a lower risk of CVD. This study found that anthocyanins and flavan-3-ols are inversely associated with risk of CVD, while flavones and flavonols with CHD [19].

Compared to these previous works, our study included nineteen studies, which is a higher number compared to Jiang et al., and Liu et al. On the other hand, our study is one of the few meta-analyses taking into consideration class-specific flavonoids for CVD in the analysis, as Micek's and Hooper's did. One of the strengths of our work is that we are analyzing CVD in early stages, where dyslipidemias and hypertension

are contributing factors that eventually lead to atherosclerosis. Therefore, our results contribute to the prevention of the development of many complications and increasing health costs that are linked to CVD. The regular consumption of flavonoids has already been proven to prevent LDLc elevations, deter the development of atherosclerosis, and ultimately CVD. Additionally, these Level 1 evidence studies on flavonoids are necessary for numerous reasons. Meta-analyses serve as checkpoints in population studies of a specific topic; summaries of state-of-the-art knowledge provide guidance to academics for future research. The evident benefit of specific flavonoids to lower TC and LDLc concentrations must be considered and further studied in order to be implemented in clinical guidelines. Currently, nutritional recommendations regarding flavonoid consumption are given to patients. However, these recommendations are not considered in clinical guidelines for the treatment of hypercholesterolemia. Lastly, clinical trials that determine specific doses and sources of pure flavonoids should be encouraged to benefit patient health.

Finally, we present antioxidant mechanisms for the most relevant flavonoids demonstrated through our analysis (Figures 6 and 7). These mechanisms apply for every flavonoid with the necessary structures to follow the reaction. The depicted example corresponds to a different class of flavonoid [43].

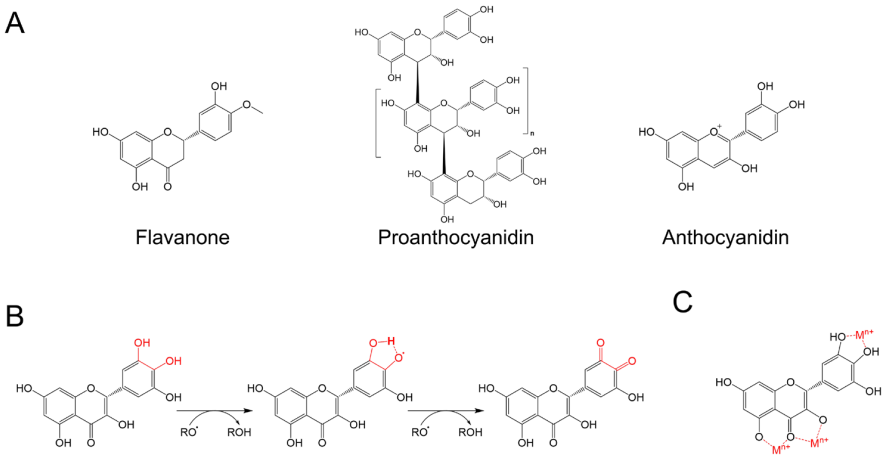

Figure 6. Classes of flavonoids with the best results to lower TC and LDLc in patients with atherosclerosis risk, and the possible mechanism of antioxidant reaction. (**A**) Flavanone, proanthocyanidin, and anthocyanidin (the anthocyanin related is the glycosil form). (**B**) Antioxidant mechanism of scavenger reactive oxygen species. (**C**) Chelation of metals.

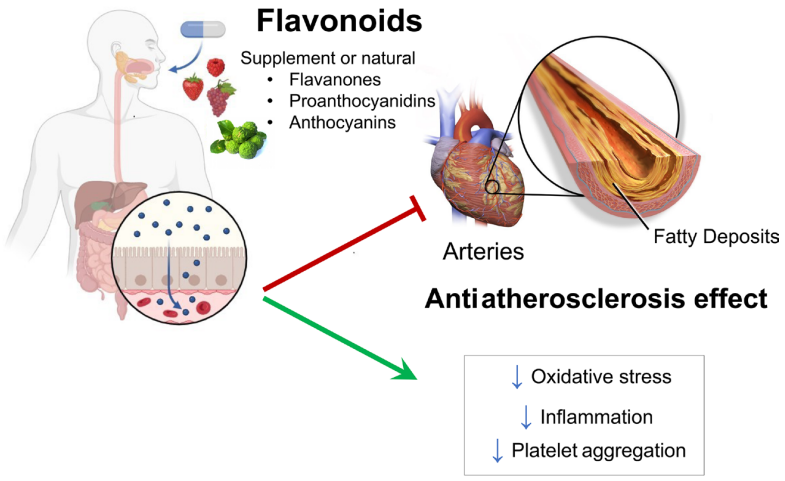

Figure 7. Benefits of specific flavonoids and their anti-atherosclerosis effects. Red line indicates inhibition and green arrow indicates activation.

5. Conclusions

Flavonoid consumption is associated with lower plasma concentrations of LDLc. These findings suggest that regular intake of flavonoids may be beneficial to reduce the aforementioned risk factor for the development of cardiovascular disease. There is evidence that the preferred flavonoids that aid in reducing LDLc are proanthocyanidins, flavanones, and anthocyanins. The examined doses were 190 mg/d, 450 mg/d to 1300 mg/d, and 320 mg/d, respectively. These findings suggest that the regular consumption of flavonoids will aid in improving LDLc plasma concentrations. Targeting risk factors with conservative management, such as lifestyle modifications, is affordable and easy to follow by patients at risk for CVD. Additionally, it aids in avoiding unpleasant drug side effects, preventing complications and increasing health costs in the future. Our analysis also demonstrates the lack of RCTs studying the effect of pure flavonoids in the different stages of atherosclerosis. Our results determine that further research for each pure flavonoid's effects is necessary to continue contributing to this promising topic, as well as the elaboration of reviews and meta-analyses that aid in defining effective doses for specific treatments. Additional RCTs are recommended with higher numbers of subjects and diverse analytic methods to obtain supplementary information that provides statistical significance. Quantitative analysis is essential to determine meaningful differences that support clinical decisions and evidence-based medicine.

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References

1. World Health Organization (WHO). Available online: https://www.who.int (accessed on 11 March 2023).
2. Chrysant, S.G. A New Paradigm in the Treatment of the Cardiovascular Disease Continuum: Focus on Prevention. *Hippokratia* **2011**, *15*, 7–11. [PubMed]
3. Libby, P. Inflammation and Cardiovascular Disease Mechanisms. *Am. J. Clin. Nutr.* **2006**, *83*, 456S–460S. [CrossRef] [PubMed]
4. Niu, Y.; Bai, N.; Ma, Y.; Zhong, P.-Y.; Shang, Y.-S.; Wang, Z.-L. Safety and Efficacy of Anti-Inflammatory Therapy in Patients with Coronary Artery Disease: A Systematic Review and Meta-Analysis. *BMC Cardiovasc. Disord.* **2022**, *22*, 84. [CrossRef] [PubMed]
5. Abdul-Rahman, T.; Bukhari, S.M.A.; Herrera, E.C.; Awuah, W.A.; Lawrence, J.; de Andrade, H.; Patel, N.; Shah, R.; Shaikh, R.; Capriles, C.A.A.; et al. Lipid Lowering Therapy: An Era Beyond Statins. *Curr. Probl. Cardiol.* **2022**, *47*, 101342. [CrossRef] [PubMed]
6. Szczepańska, E.; Białek-Dratwa, A.; Janota, B.; Kowalski, O. Dietary Therapy in Prevention of Cardiovascular Disease (CVD)-Tradition or Modernity? A Review of the Latest Approaches to Nutrition in CVD. *Nutrients* **2022**, *14*, 2649. [CrossRef]
7. Sharif, H.; Akash, M.S.H.; Rehman, K.; Irshad, K.; Imran, I. Pathophysiology of Atherosclerosis: Association of Risk Factors and Treatment Strategies Using Plant-Based Bioactive Compounds. *J. Food Biochem.* **2020**, *44*, e13449. [CrossRef]
8. Hoensch, H.P.; Oertel, R. The Value of Flavonoids for the Human Nutrition: Short Review and Perspectives. *Clin. Nutr. Exp.* **2015**, *3*, 8–14. [CrossRef]

9. Pangestika, I.; Oksal, E.; Tengku Muhammad, T.S.; Amir, H.; Syamsumir, D.F.; Wahid, M.E.A.; Andriani, Y. Inhibitory effects of tangeretin and trans-ethyl caffeate on the HMG-CoA reductase activity: Potential agents for reducing cholesterol levels. Saudi Journal of Biological Sciences. *Saudi J. Biol. Sci.* **2020**, *27*, 1947–1960. [CrossRef]
10. Ma, C.; Zhang, J.; Yang, S.; Hua, Y.; Su, J.; Shang, Y.; Fan, G. Astragalus Flavone Ameliorates Atherosclerosis and Hepatic Steatosis via Inhibiting Lipid-Disorder and Inflammation in apoE-/- Mice. *Front. Pharmacol.* **2020**, *11*, 610550. [CrossRef]
11. Georgiev, V.; Ananga, A.; Tsolova, V. Recent Advances and Uses of Grape Flavonoids as Nutraceuticals. *Nutrients* **2014**, *6*, 391–415. [CrossRef]
12. Côrtes, S.F.; Rezende, B.A.; Corriu, C.; Medeiros, I.A.; Teixeira, M.M.; Lopes, M.J.; Lemos, V.S. Pharmacological Evidence for the Activation of Potassium Channels as the Mechanism Involved in the Hypotensive and Vasorelaxant Effect of Dioclein in Rat Small Resistance Arteries. *Br. J. Pharmacol.* **2001**, *133*, 849–858. [CrossRef]
13. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; et al. Phenol-Explorer: An Online Comprehensive Database on Polyphenol Contents in Foods. *Database* **2010**, *2010*, bap024. [CrossRef] [PubMed]
14. Esmaillzadeh, A.; Azadbakht, L. Major Dietary Patterns in Relation to General Obesity and Central Adiposity among Iranian Women. *J. Nutr.* **2008**, *138*, 358–363. [CrossRef] [PubMed]
15. Habauzit, V.; Verny, M.-A.; Milenkovic, D.; Barber-Chamoux, N.; Mazur, A.; Dubray, C.; Morand, C. Flavanones Protect from Arterial Stiffness in Postmenopausal Women Consuming Grapefruit Juice for 6 Mo: A Randomized, Controlled, Crossover Trial. *Am. J. Clin. Nutr.* **2015**, *102*, 66–74. [CrossRef] [PubMed]
16. Hooper, L.; Kroon, P.A.; Rimm, E.B.; Cohn, J.S.; Harvey, I.; Le Cornu, K.A.; Ryder, J.J.; Hall, W.L.; Cassidy, A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **2008**, *88*, 38–50. [CrossRef] [PubMed]
17. Jiang, W.; Wei, H.; He, B. Dietary flavonoids intake and the risk of coronary heart disease: A dose-response meta-analysis of 15 prospective studies. *Thromb. Res.* **2015**, *135*, 459–463. [CrossRef] [PubMed]
18. Liu, X.M.; Liu, Y.J.; Huang, Y.; Yu, H.J.; Yuan, S.; Tang, B.W.; Wang, P.G.; He, Q.Q. Dietary total flavonoids intake and risk of mortality from all causes and cardiovascular disease in the general population: A systematic review and meta-analysis of cohort studies. *Mol. Nutr. Food Res.* **2017**, *61*, 6. [CrossRef] [PubMed]
19. Micek, A.; Godos, J.; Del Rio, D.; Galvano, F.; Grosso, G. Dietary Flavonoids and Cardiovascular Disease: A Comprehensive Dose-Response Meta-Analysis. *Mol. Nutr. Food Res.* **2021**, *65*, e2001019. [CrossRef]
20. Thiriet, C.; Mahjoub, K.; Courte, G.; Labroca, P.; Cravoisy, A.; Lemarie, J.; Conrad, M.; Nace, L.; Bollaert, P.-E.; Gibot, S. Automated Measurement of Neutrophil CD64 Expression for Diagnosing Sepsis in Critically Ill Patients. *Minerva Anestesiol.* **2019**, *85*, 943–950. [CrossRef]
21. Zhu, Y.; Xia, M.; Yang, Y.; Liu, F.; Li, Z.; Hao, Y.; Mi, M.; Jin, T.; Ling, W. Purified Anthocyanin Supplementation Improves Endothelial Function via NO-CGMP Activation in Hypercholesterolemic Individuals. *Clin. Chem.* **2011**, *57*, 1524–1533. [CrossRef]
22. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* **2021**, *372*, n71. [CrossRef]
23. R: A Language and Environment for Statistical Computing. Available online: https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing (accessed on 30 August 2023).
24. Borenstein, M.; Hedges, L.V.; Higgins, J.P.T.; Rothstein, H.R. *Introduction to Meta-Analysis*; John Wiley & Sons: Hoboken, NJ, USA, 2021; ISBN 978-1-119-55838-5.
25. Samman, S.; Lyons Wall, P.M.; Chan, G.S.; Smith, S.J.; Petocz, P. The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. *Atherosclerosis* **1999**, *147*, 277–283. [CrossRef] [PubMed]
26. Wang, Y.; Jones, P.J.; Ausman, L.M.; Lichtenstein, A.H. Soy protein reduces triglyceride levels and triglyceride fatty acid fractional synthesis rate in hypercholesterolemic subjects. *Atherosclerosis* **2004**, *173*, 269–275. [CrossRef]
27. Hodgson, J.M.; Burke, V.; Puddey, I.B. Acute Effects of Tea on Fasting and Postprandial Vascular Function and Blood Pressure in Humans. *J. Hypertens.* **2005**, *23*, 47–54. [CrossRef] [PubMed]
28. Wang-Polagruto, J.F.; Villablanca, A.C.; Polagruto, J.A.; Lee, L.; Holt, R.R.; Schrader, H.R.; Ensunsa, J.L.; Steinberg, F.M.; Schmitz, H.H.; Keen, C.L. Chronic Consumption of Flavanol-Rich Cocoa Improves Endothelial Function and Decreases Vascular Cell Adhesion Molecule in Hypercholesterolemic Postmenopausal Women. *J. Cardiovasc. Pharmacol.* **2006**, *47* (Suppl. S2), S177–S186. [CrossRef] [PubMed]
29. Nestel, P.; Fujii, A.; Zhang, L. An Isoflavone Metabolite Reduces Arterial Stiffness and Blood Pressure in Overweight Men and Postmenopausal Women. *Atherosclerosis* **2007**, *192*, 184–189. [CrossRef] [PubMed]
30. Widlansky, M.E.; Hamburg, N.M.; Anter, E.; Holbrook, M.; Kahn, D.F.; Elliott, J.G.; Keaney, J.F.; Vita, J.A. Acute EGCG Supplementation Reverses Endothelial Dysfunction in Patients with Coronary Artery Disease. *J. Am. Coll. Nutr.* **2007**, *26*, 95–102. [CrossRef] [PubMed]
31. Qin, Y.; Xia, M.; Ma, J.; Hao, Y.; Liu, J.; Mou, H.; Cao, L.; Ling, W. Anthocyanin Supplementation Improves Serum LDL- and HDL-Cholesterol Concentrations Associated with the Inhibition of Cholesteryl Ester Transfer Protein in Dyslipidemic Subjects. *Am. J. Clin. Nutr.* **2009**, *90*, 485–492. [CrossRef]

32. Pfeuffer, M.; Auinger, A.; Bley, U.; Kraus-Stojanowic, I.; Laue, C.; Winkler, P.; Rüfer, C.E.; Frank, J.; Bösch-Saadatmandi, C.; Rimbach, G.; et al. Effect of Quercetin on Traits of the Metabolic Syndrome, Endothelial Function and Inflammation in Men with Different APOE Isoforms. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 403–409. [CrossRef]
33. Zhu, Y.; Ling, W.; Guo, H.; Song, F.; Ye, Q.; Zou, T.; Li, D.; Zhang, Y.; Li, G.; Xiao, Y.; et al. Anti-Inflammatory Effect of Purified Dietary Anthocyanin in Adults with Hypercholesterolemia: A Randomized Controlled Trial. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 843–849. [CrossRef]
34. Koutelidakis, A.E.; Rallidis, L.; Koniari, K.; Panagiotakos, D.; Komaitis, M.; Zampelas, A.; Anastasiou-Nana, M.; Kapsokefalou, M. Effect of green tea on postprandial antioxidant capacity, serum lipids, C-reactive protein and glucose levels in patients with coronary artery disease. *Eur. J. Nutr.* **2014**, *53*, 479–486. [CrossRef] [PubMed]
35. Davinelli, S.; Bertoglio, J.C.; Zarrelli, A.; Pina, R.; Scapagnini, G. A Randomized Clinical Trial Evaluating the Efficacy of an Anthocyanin-Maqui Berry Extract (Delphinol®) on Oxidative Stress Biomarkers. *J. Am. Coll. Nutr.* **2015**, *34* (Suppl. S1), 28–33. [CrossRef]
36. Johnson, S.A.; Figueroa, A.; Navaei, N.; Wong, A.; Kalfon, R.; Ormsbee, L.T.; Feresin, R.G.; Elam, M.L.; Hooshmand, S.; Payton, M.E.; et al. Daily blueberry consumption improves blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension: A randomized, double-blind, placebo-controlled clinical trial. *J. Acad. Nutr. Diet.* **2015**, *115*, 369–377. [CrossRef] [PubMed]
37. Zhang, X.; Zhu, Y.; Song, F.; Yao, Y.; Ya, F.; Li, D.; Ling, W.; Yang, Y. Effects of Purified Anthocyanin Supplementation on Platelet Chemokines in Hypocholesterolemic Individuals: A Randomized Controlled Trial. *Nutr. Metab.* **2016**, *13*, 86. [CrossRef]
38. Argani, H.; Ghorbanihaghjo, A.; Vatankhahan, H.; Rashtchizadeh, N.; Raeisi, S.; Ilghami, H. The Effect of Red Grape Seed Extract on Serum Paraoxonase Activity in Patients with Mild to Moderate Hyperlipidemia. *Sao Paulo Med. J.* **2016**, *134*, 234–239. [CrossRef] [PubMed]
39. Nogueira, L.P.; Nogueira, J.F.; Klein, M.R.S.T.; Sanjuliani, A.F. Short-term Effects of Green Tea on Blood Pressure, Endothelial Function, and Metabolic Profile in Obese Prehypertensive Women: A Crossover Randomized Clinical Trial. *J. Am. Coll. Nutr.* **2016**, *36*, 108–115. [CrossRef]
40. Salden, B.N.; Troost, F.J.; de Groot, E.; Stevens, Y.R.; Garcés-Rimón, M.; Possemiers, S.; Winkens, B.; Masclee, A.A. Randomized Clinical Trial on the Efficacy of Hesperidin 2S on Validated Cardiovascular Biomarkers in Healthy Overweight Individuals. *Am. J. Clin. Nutr.* **2016**, *104*, 1523–1533. [CrossRef] [PubMed]
41. Hollands, W.J.; Tapp, H.; Defernez, M.; Perez Moral, N.; Winterbone, M.S.; Philo, M.; Lucey, A.J.; Kiely, M.E.; Kroon, P.A. Lack of Acute or Chronic Effects of Epicatechin-Rich and Procyanidin-Rich Apple Extracts on Blood Pressure and Cardiometabolic Biomarkers in Adults with Moderately Elevated Blood Pressure: A Randomized, Placebo-Controlled Crossover Trial. *Am. J. Clin. Nutr.* **2018**, *108*, 1006–1014. [CrossRef]
42. Capomolla, A.S.; Janda, E.; Paone, S.; Parafati, M.; Sawicki, T.; Mollace, R.; Ragusa, S.; Mollace, V. Atherogenic Index Reduction and Weight Loss in Metabolic Syndrome Patients Treated with A Novel Pectin-Enriched Formulation of Bergamot Polyphenols. *Nutrients* **2019**, *11*, 1271. [CrossRef]
43. Grijalva-Guiza, R.E.; Jiménez-Garduño, A.M.; Hernández, L.R. Potential Benefits of Flavonoids on the Progression of Atherosclerosis by Their Effect on Vascular Smooth Muscle Excitability. *Molecules* **2021**, *26*, 3557. [CrossRef]
44. Weggemans, R.M.; Trautwein, E.A. Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: A meta-analysis. *Eur. J. Clin. Nutr.* **2003**, *57*, 940–946. [CrossRef] [PubMed]
45. Zhuo, X.G.; Melby, M.K.; Watanabe, S. Soy isoflavone intake lowers serum LDL cholesterol: A meta-analysis of 8 randomized controlled trials in humans. *J. Nutr.* **2004**, *134*, 2395–2400. [CrossRef] [PubMed]
46. Taku, K.; Umegaki, K.; Sato, Y.; Taki, Y.; Endoh, K.; Watanabe, S. Soy isoflavones lower serum total and LDL cholesterol in humans: A meta-analysis of 11 randomized controlled trials. *Am. J. Clin. Nutr.* **2007**, *85*, 1148–1156, Correction in *Am. J. Clin. Nutr.* **2007**, *86*, 809.. [CrossRef] [PubMed]
47. Raman, G.; Avendano, E.E.; Chen, S.; Wang, J.; Matson, J.; Gayer, B.; Novotny, J.A.; Cassidy, A. Dietary Intakes of Flavan-3-Ols and Cardiometabolic Health: Systematic Review and Meta-Analysis of Randomized Trials and Prospective Cohort Studies. *Am. J. Clin. Nutr.* **2019**, *110*, 1067–1078. [CrossRef]
48. Serban, M.-C.; Sahebkar, A.; Zanchetti, A.; Mikhailidis, D.P.; Howard, G.; Antal, D.; Andrica, F.; Ahmed, A.; Aronow, W.S.; Muntner, P.; et al. Effects of Quercetin on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J. Am. Heart Assoc.* **2016**, *5*, e002713. [CrossRef]
49. Daneshzad, E.; Shab-Bidar, S.; Mohammadpour, Z.; Djafarian, K. Effect of Anthocyanin Supplementation on Cardio-Metabolic Biomarkers: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Clin. Nutr.* **2019**, *38*, 1153–1165. [CrossRef]
50. Sone, T.; Kuriyama, S.; Nakaya, N.; Hozawa, A.; Shimazu, T.; Nomura, K.; Rikimaru, S.; Tsuji, I. Randomized Controlled Trial for an Effect of Catechin-Enriched Green Tea Consumption on Adiponectin and Cardiovascular Disease Risk Factors. *Food Nutr. Res.* **2011**, *55*, 8326. [CrossRef]