RESEARCH ARTICLE

Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products

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Scope: Previous systematic reviews suggest beneficial effects of flavonoids on biomarkers of cardiovascular disease (CVD) risk, but have overlooked the impact of dose response or food complexity. The aim of the present study was to examine the relative impact of composition, flavonoid structure and dose.

Methods and results: MEDLINE, EMBASE and Cochrane were searched for randomised controlled trials (RCTs) of flavonoids or flavonoid-rich foods/extracts. Flavonoid composition was established using United States Department of Agriculture (USDA) and Phenol-Explorer databases. Effects of six flavonoid subgroups on endothelial function (flow-mediated dilation; FMD), and systolic and diastolic blood pressures were assessed by random effects meta-analyses and regression analyses. Meta-analyses of combined flavonoid subclasses showed significant improvements in FMD (chronic, 0.73% (0.17, 1.30) 14 RCTs; acute, 2.33% (1.58, 3.08) 18 RCTs) and blood pressures (systolic, –1.46 mmHg (–2.38, –0.53) 63 RCTs; diastolic, –1.25 mmHg (–1.82, –0.67) 63 RCTs). Similar benefits were observed for the flavan-3-ol, catechol flavonoids (catechins, quercetin, cyanidin etc.), procyanidins, epicatechin and catechin subgroups. Dose-response relationships were non-linear for FMD ($R^2 \leq 0.30$), with greater associations observed when applying polynomial regression analyses ($R^2 \leq 0.72$); there was no indication of a dose response for blood pressure.

Conclusion: The present analysis suggests that flavonoid bioactivity does not follow a classical linear dose-response association and this may have important biological implications.

Keywords:
Blood pressure / Cardiovascular disease / Dose response / Flavonoids / Flow-mediated dilation

1 Introduction

Evidence from epidemiological studies and randomised controlled trials (RCTs) together with in vitro data on vascular bioactivity support a potential role for some flavonoids in the reduction in risk of cardiovascular disease [1–3]. Although numerous short-term RCTs have been published to date, their findings have often been inconsistent, most likely as studies have often been idealistically designed and/or interpreted. Intervention studies on plant-derived food products are particularly complex as the content of plant bioactives fed are chemically diverse and extremely variable relative to pure preparations. Limitations in data reporting have also made systematically reviewing the data challenging. For many RCTs, there has been inadequate assessment of flavonoid composition of either the foods fed or in analyses of the biological samples, limited dose response analysis and

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Abbreviations: BP, blood pressure; **DBP**, diastolic blood pressure; **FMD**, flow-mediated dilation; **RCT**, randomised controlled trials; **SBP**, systolic blood pressure

inconsistencies in biomarkers measured across studies [4, 5]. In order to further progress in our understanding of the bioactivity of flavonoids, these potential limitations need to be explored.

Flavonoids are a diverse group of polyphenolic compounds that have been traditionally subdivided into six major subclasses (Table 1). Previous meta-analyses have evaluated the effects of flavonoids based on a single flavonoid subclass within a food, such as flavan-3-ols (catechins) in chocolate, anthocyanins in wine and quercetin in onions [2, 6–8]. However, this approach does not take into account the complex array of flavonoid compounds present within any given food product. For example, red wine is commonly identified as a rich source of anthocyanins; however, wine contains a complex array of flavonoids, and some wines may contain equivalent or greater amounts (mg/100 g) of flavan-3-ols over anthocyanins [9]. Similarly, for many foods (e.g. tea, wine and chocolate), polymeric flavonoids such as tannins or thearubigins (which are composed of repeating flavonoid units) are found in much higher concentrations than all the monomeric forms (which are composed of one flavonoid) combined [9–11]. Therefore, dietary interventions are often misclassified as providing one flavonoid source over another, thus obscuring the effects in meta-analysis. The present review compares the conventional strategy of reporting the biological effect of one flavonoid constituent within a food product, with one which utilises available flavonoid databases to examine the impact of total flavonoid composition, chemical structure and dose, including polymer and monomer content (Table 1). The aim of the present study was to examine the relative impact of composition, flavonoid structure and dose, with the objective of highlighting gaps in the present literature, developing new insights into flavonoid bioactivity and new hypotheses for future investigation.

2 Materials and methods

We included RCTs of parallel or crossover design that randomised adult humans to a nutrition intervention involving flavonoids or flavonoid-rich foods/extracts compared to a control group, and which reported effects on established or emerging cardiovascular risk biomarkers; using methods as previously published [8] (Supporting Information Appendix 1). Briefly, our primary outcomes were flow-mediated dilation (FMD) and blood pressure (BP) and we searched MEDLINE, EMBASE and the Cochrane Library databases. All included abstracts were identified independently by two reviewers and data were extracted independently from all included studies with discrepancies (if any) adjudicated by a third reviewer.

The composition of each intervention included in these analyses was individually extracted from each manuscript, when present, and if no compositional data were provided, it was established using comprehensive online flavonoid databases [9, 10]. Data on 26 flavonoid species across six subclasses were initially extracted for the interventions used in each study (Table 1) using the USDA flavonoid database [10], with Phenol-Explorer used where data were incomplete or missing [9]. A more detailed description of the methods utilised for establishing the flavonoid compositions of the included RCTs is provided as Supporting Information Appendix 2. This led to six intervention classifications based on unique compositional characteristics of the flavonoid-rich food/extract fed in the intervention studies (further details regarding trial classifications is provided in the Supporting Information Appendix 3). Briefly, studies were classified by total flavonoids (representing a summation of all flavonoids within the RCTs) and the most abundant individual subclass constituent (both including and excluding polymers), as well as by unique B- and C-ring constituent structures; where total cumulative dose was established for each.

Random effects meta-analyses (using Review Manager (RevMan) Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011) were carried out for total flavonoids or most abundant flavonoid or subclass (Supporting Information Appendix 3) versus control, for the three primary outcomes (FMD, systolic blood pressure (SBP) and diastolic blood pressure (DBP)) including all relevant RCTs, where a statistically significant effect of flavonoids or a subclass was seen. The dose response was explored via linear and polynomial regression analyses in Excel (Microsoft Excel; Microsoft Corp., Seattle, WA).

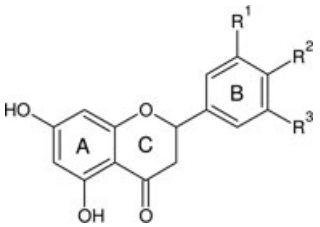
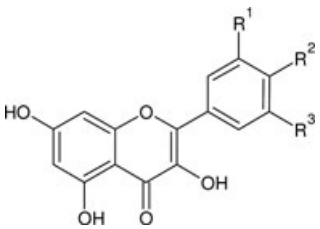
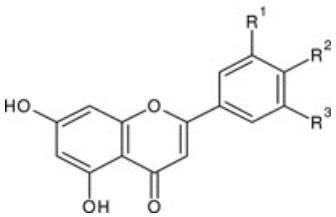
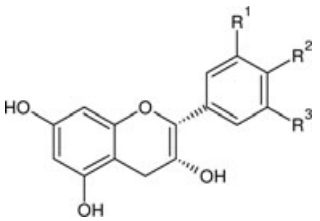
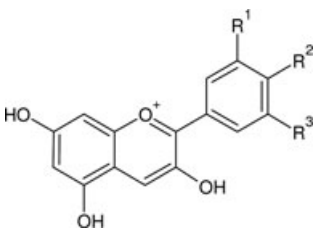
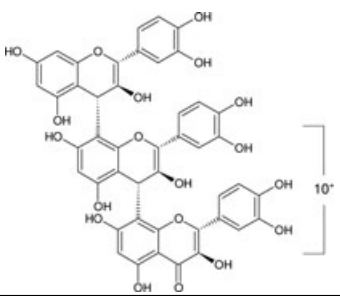
3 Results

3.1 Data extraction

We assessed 8893 titles and abstracts for inclusion in the systematic review. Of these, 487 were collected as full text and assessed for inclusion. Of these, 184 intervention studies were included for composition extraction. Compositional analysis for establishing dose response was possible for 174 studies (Supporting Information Appendices 4 and 5). Of these studies, 63 provided data on BP, 32 on FMD, with 77 interventions included in total (as some reported both FMD and BP).

Of the included intervention studies, where compositional analyses were possible, unique meta-analysis for total flavonoids, most abundant subclass (C- and B-ring classification) and most abundant subclass constituent (including and excluding polymers) was performed (description of the individual RCT subclassifications is provided as Supporting Information Appendices 5 and 6). The majority of included interventions fed chocolate (20%), tea (21% green, 9% black), red wine (15%) and berries or grapes (15%) and most included studies were interventions involving the flavan-3-ol subclass (51% of studies), catechol/dihydroxy flavonoids (68% of studies) or the flavan-3-ol subclass constituent epigallocatechin gallate (15%). When we included polymers in the analyses, 51% of the studies were classified as flavonoid polymer

Table 1. Structural configuration of common dietary flavonoids: subclass, subclass constituent and B-ring configuration

Flavonoid (subclass)	Subclass structure	Subclass constituent	B-ring configuration[a)]		
			R^1	R^2	R^3
Flavanones		Naringenin	H	OH	H
		Eriodictyol	OH	OH	H
		Hesperetin	OH	O-CH_3	H
		Taxifolin	OH	OH	H
Flavonols		Kaempferol	H	OH	H
		Quercetin	OH	OH	H
		Myricetin	OH	OH	OH
		Isorhamnetin	O-CH_3	OH	H
Flavones		Apigenin	H	OH	H
		Luteolin	OH	OH	H
		Tricetin	OH	OH	OH
		Chrysoeriol	O-CH_3	OH	H
Flavan-3-ols		Epicatechin	OH	OH	H
		Epicatechin gallate	OH	OH	H
		Epigallocatechin	OH	OH	OH
		Epigallocatechin gallate	OH	OH	OH
Anthocyanins/anthocyanidins		Cyanidin	OH	OH	H
		Delphinidin	OH	OH	OH
		Peonidin	O-CH_3	OH	H
		Pelargonidin	H	OH	H
		Malvidin	O-CH_3	OH	O-CH_3
		Petunidin	O-CH_3	OH	OH
Polymers[b)]		Procyanidins	NA	NA	NA
		Tannins	NA	NA	NA
		Theoflavins	NA	NA	NA

a) R^{1-3}, functional group identifier.
b) Structure provided for the polymer subclass is one example of a polyphenol polymer; however, polyphenol polymers can have tremendous structural diversity.
OH, hydroxyl conjugate; O-CH_3, methoxly conjugate; polymer, composed of repeating structural units.

Table 2. Dose characteristics of the flavonoid constituents utilised in the intervention studies

Subgrouping[a)]	Proportion (as %) of interventions versus dose (mg/day)	
Total flavonoids (monomeric and polymeric)	68% ≤ 500	34% ≤ 200
Total flavonoids (monomeric only)	65% ≤ 200	38% ≤ 100
	Mean and maximum dose of subclass constituents	
	Mean (mg/day, ±SD)	Maximum (mg/day)
Polymers	369 ± 367	2080
Epigallocatechin gallate	259 ± 222	1038
Quercetin	512 ± 438	1000
Catechin	67 ± 55	149
Cyanidin	175 ± 171	500
Epicatechin	159 ± 190	556

a) Subgroupings include total flavonoid monomers, total flavonoid monomers and polymers together and individual flavonoid subclasses (e.g. polymers, epigallocatechin gallate, quercetin, catechin, cyanidin and epicatechin).

interventions. In the analysis of dose response, the majority of interventions fed well below 500 mg/day of total polymeric and monomeric constituents combined, and/or 200 mg/day monomeric constituents (Table 2).

3.2 FMD response

In pooled analysis of all flavonoid interventions (including both monomeric and polymeric forms), FMD improved both acutely (2.33% (1.58, 3.68)) and chronically (0.73% (0.17, 1.30)) (Fig. 1A and B, respectively). When we further examined subclasses and individual constituents, the magnitude of the FMD response was greater for several flavonoid subclass constituents (Table 3). In particular, the acute FMD responses for epicatechin, catechin or procyanidins ranged from 3.22 to 3.38%, compared to analyses that grouped subclasses together, such as total flavonoids, flavan-3-ols and catechol flavonoids (ranging from 2.33 to 2.81%; Table 3A). The responses for chronic FMD followed a similar pattern (Table 3B), although the magnitude of the acute FMD effect was greater (ranging from 2.33 to 3.38 % for acute FMD versus 0.73 to 2.32% for chronic FMD).

3.3 BP response

When all flavonoid interventions were pooled, there was a significant reduction in BP observed; SBP (–1.46 mmHg (−2.38, −0.53)) and DBP (–1.25 mmHg (−1.82, −0.67)) (Fig. 2). Similar to the effects observed for the acute FMD response (Table 3A), BP response was greatest for epicatechin, quercetin and procyanidins (−1.67 to −2.36 mmHg), compared to analyses that grouped subclass constituents together,

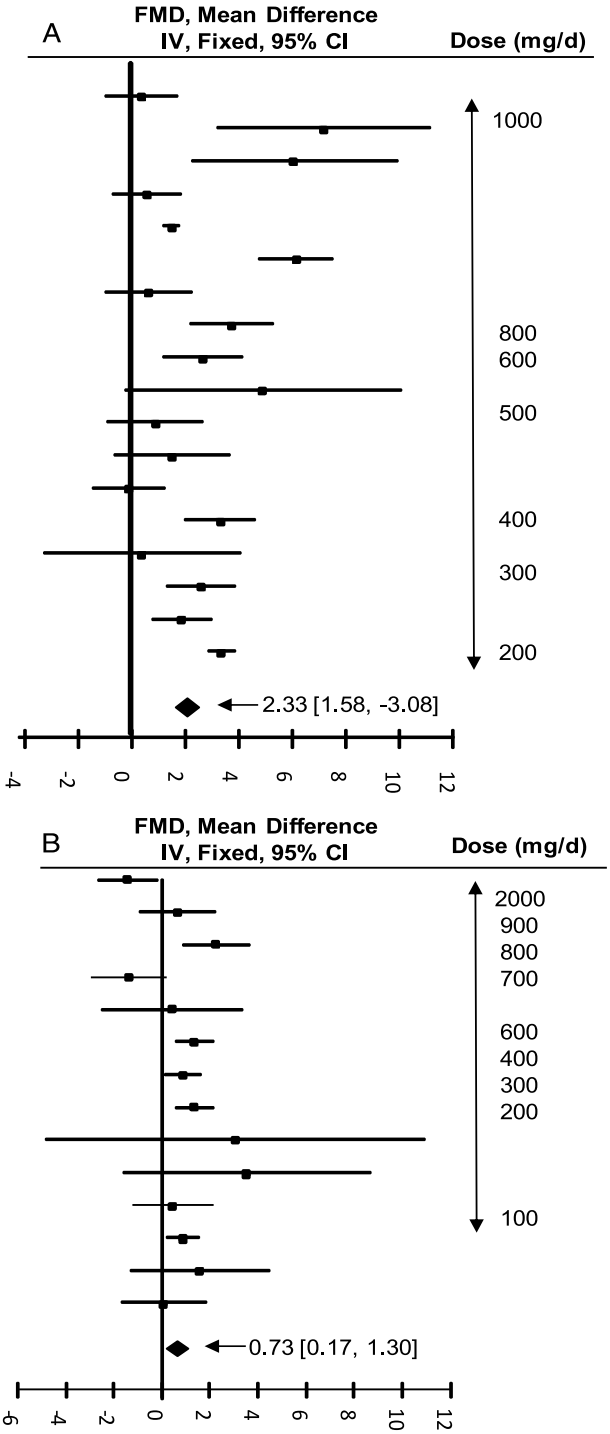


Figure 1. FMD response to all flavonoid interventions (total flavonoids: monomeric and polymeric forms). Data are presented (as % change) for random effects meta-analysis conducted using inverse variance for A, acute (90–150 min) FMD response ($n = 18$) and B, chronic (≥2 weeks intervention) FMD response ($n = 14$). FMD, flow-mediated dilation.

Table 3. The effect of flavonoids (class), flavonoid subclass and subclass constituents on FMD and BP[a)]

Risk factor	Subgroup analysis	Mean effect (%)	*n*	I^2 (%)	*p*-value
(A) FMD acute[b)]	Total flavonoids	2.33 [1.58, 3.08]	18	86	$p < 0.00001$
	Flavan-3-ols	2.81 [1.92, 3.69]	13	88	$p \leq 0.00001$
	Catechol Flavonoids	2.47 [1.67, 3.28]	16	88	$p \leq 0.00001$
	Procyanidins	3.38 [2.19, 4.58]	9	92	$p \leq 0.00001$
	Epicatechin	3.22 [1.94, 4.50]	9	88	$p \leq 0.00001$
	Catechin	3.22 [2.66, 3.78]	2	15	$p < 0.00001$
(B) FMD Chronic[b)]	Total flavonoids	0.73 [0.17, 1.30]	14	58	$p = 0.01$
	Flavan-3-ols	1.03 [0.58, 1.48]	12	30	$p \leq 0.00001$
	Catechol flavonoids	0.75 [0.15, 1.35]	13	61	$p \leq 0.01$
	Procyanidins	1.09 [0.56, 1.61]	10	40	$p < 0.0001$
	Epicatechin	0.94 [0.47, 1.42]	9	32	$p = 0.0001$
	Catechin	2.32 [0.97, 3.68]	2	0	$p = 0.00008$
(C) Chronic SBP[c)]	Total flavonoids	−1.46 [−2.38, −0.53]	63	80	$p = 0.002$
	Flavan-3-ols	−1.50 [−3.01, 0.01]	37	84	$p = 0.05$
	Catechol flavonoids	−1.69 [−2.80, −0.58]	45	83	$p = 0.003$
	Procyanidins	−2.33 [−3.81, −0.85]	26	84	$p = 0.002$
	Epicatechin	−2.36 [−4.54, −0.18]	21	89	$p = 0.03$
(D) Chronic DBP[c)]	Total flavonoids	−1.25 [−1.82, −0.67]	63	70	$p \leq 0.0001$
	Flavan-3-ols	−1.24 [−2.00, −0.49]	38	65	$p = 0.001$
	Catechol flavonoids	−1.24 [−1.89, −0.58]	45	70	$p = 0.0002$
	Procyanidins	−1.83 [−2.82, −0.85]	26	77	$p \leq 0.0003$
	Epicatechin	−1.67 [−2.76, −0.58]	21	72	$p = 0.003$
	Quercetin	−1.76 [−3.54, 0.02]	5	40	$p = 0.05$

Class, total flavonoids; subclass, flavan-3-ols and catechol flavonoids; subclass constituents, procyanidins, epicatechin, catechin and quercetin.
a) Meta-analyses utilised fixed effects mean differences, to allow assessment of *p*-value for difference between subgroups.
b) Inverse variance, mean difference IV, Random, 95% CI.
c) mmHg, IV Random, 95% CI.

such as total flavonoids, flavan-3-ols or catechol flavonoids (−1.24 to −1.69 mmHg) (Tables 3C and 3D).

3.4 Dose response

There was no indication of a linear dose response ($R^2 < 0.17$) for acute FMD across the entire dose range for total flavonoids (Fig. 3A and B), flavan-3-ols (Fig. 3C), catechol flavonoids (Fig. 3D), procyanidins (Fig. 3E) or epicatechin (Fig. 3F). Non-linear dose-response associations (inverted U-shaped and bimodal) were indicated in polynomial regression analyses for total flavonoids ($R^2 = 0.20$), flavan-3-ols ($R^2 = 0.28$), procyanidins ($R^2 = 0.51$) and epicatechin ($R^2 = 0.31$); where FMD response increased in magnitude with increasing dose only at the lower intake levels (e.g. doses <1 g/day for total flavonoids and procyanidins or doses <200 mg/day for monomeric forms). There was also no suggestion of a linear dose response ($R^2 < 0.13$) for chronic FMD across the entire dose range for total flavonoids (Fig. 4A), flavan-3-ols (Fig. 3C), procyanidins (Fig. 3E) or epicatechin (Fig. 3F), but a linear dose response was observed for total monomeric flavonoids ($R^2 = 0.23$) and catechol flavonoids ($R^2 = 0.3$), where FMD response decreased with increasing flavonoid dose. Similar to acute FMD response, non-linear dose-response associations were observed in polynomial regression analyses for total flavonoids ($R^2 > 0.34$), flavan-3-ols ($R^2 = 0.26$), catechol flavonoids ($R^2 = 0.33$), procyanidins ($R^2 = 0.72$) and epicatechin ($R^2 = 0.59$); where inverted U-shaped, U-shaped and bimodal dose-responses were indicated.

For both SBP and DBP, there was no evidence of either a linear or non-linear dose-response relationship ($p < 0.07$ and $p < 0.17$, respectively) across the entire dose range for flavonoid class, subclass or subclass constituent (Figs. 5 and 6).

4 Discussion

Our data reinforce the findings from previous systematic reviews [2, 6, 8, 12, 13] regarding the beneficial effects of specific flavonoid subclasses on the acute and chronic FMD response. On the basis of our data, flavan-3-ols, catechol (B-ring) flavonoids, procyanidins and epicatechin appear to exert this effect to varying extents, suggesting effects on FMD may not be confined to a single flavonoid subclass or food source. From the present data set it was not possible to make inferences regarding the specific compositional breakdown of the polymers within the intervention foods, largely because both RCTs and food composition databases currently lack this level of detail. In addition, it is not possible from this data set to establish if the effects observed for procyanidins are the result of the procyanidins themselves or monomeric species within procyanidin-rich foods.

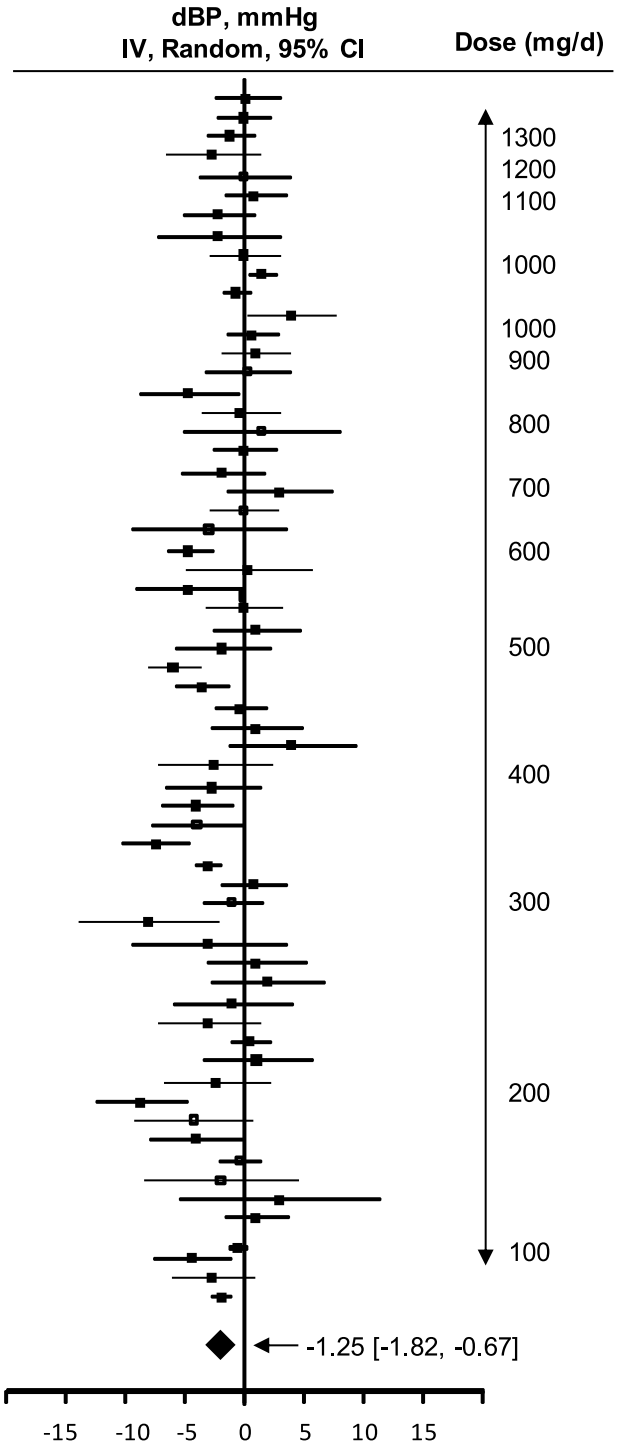


Figure 2. Diastolic blood pressure (DBP) response ($n = 63$) to all flavonoid interventions (total monomeric and polymeric flavonoids). Data are presented (as change in mmHg) for random effects meta-analysis inverse variance for chronic (≥2 weeks intervention) diastolic blood pressure response (mmHg).

Crucially, our data suggest that some classes of flavonoids appear to have differential magnitudes of biological activity depending on the dose ingested, and specifically for flavan-3-ols, catechol flavonoids, procyanidins and epicatechin. Although none of these present subgroupings are distinctively unique, our data set suggests that the effect of different subclasses of flavonoids may differ across intake level for FMD and BP. For example, there was little indication of a dose response across the entire dose range (1–2.6 g/day) for FMD when flavonoid interventions were grouped together (monomers and polymers); however, there were indications of dose-responses within defined dose ranges (e.g. below 200 mg/day for flavonoid monomers and 1 g/day for total flavonoids and flavonoid polymers) within some of the flavonoid subclassifications. Specifically, acute FMD response appeared to increase with increasing doses of procyanidins at doses below 500 mg/day and catechol flavonoids below 200 mg/day. In addition chronic FMD response appeared to increase with increasing dose for procyanidins at doses up to 400 mg/day and above 600 mg/day and flavan-3-ols and catechol flavonoids at doses below 100 mg/day.

In line with previous systematic reviews [2, 6, 12], we also observed an overall beneficial effect of flavonoids on BP and our data suggest that this relationship is particularly consistent for the flavan-3-ols and the catechol B-ring flavonoids as subclasses, and procyanidins and epicatechin as subclass constituents. There was little evidence suggesting that this effect was dose-dependent. An unexpected finding was that improvements in BP (both SBP and DBP) were strongest at lower doses and not apparent at the highest doses; however, this relationship between flavonoids on BP has been reported in tea interventions previously [14]. Together these data suggest that in addition to distinguishing between the effects of polyphenol polymers relative to monomers in future studies, it is imperative that interventions also explore dose response.

The present systematic review indicates that few 'high-dose' flavonoid interventions have been conducted to date (i.e. >500 mg/day total polymeric and monomeric forms or >200 mg/day total monomeric forms); which has left the present interpretation of dose response somewhat incomplete. The present graphical indications of inverted U-shaped, U-shaped and bimodal dose responses should therefore be interpreted with caution. In addition, the majority of flavonoid interventions conducted to date have primarily fed foods in which polyphenol polymers and catechol B-ring flavonoids are the predominant forms (>50%). Therefore, it is important that future studies establish the effects for polymers and monomers across a large range of doses (and particularly at higher doses), in addition to establishing the effects of polymeric versus monomeric flavonoids in pure forms and in foods. Future studies should also consider the involvement of 'non-flavonoid' phenolic compounds (e.g. cinnamates and chlorogenic acids) that often contribute substantially to the composition of flavonoid-rich foods. Importantly, these future RCTs must also provide complete detailed flavonoid compositional analysis.

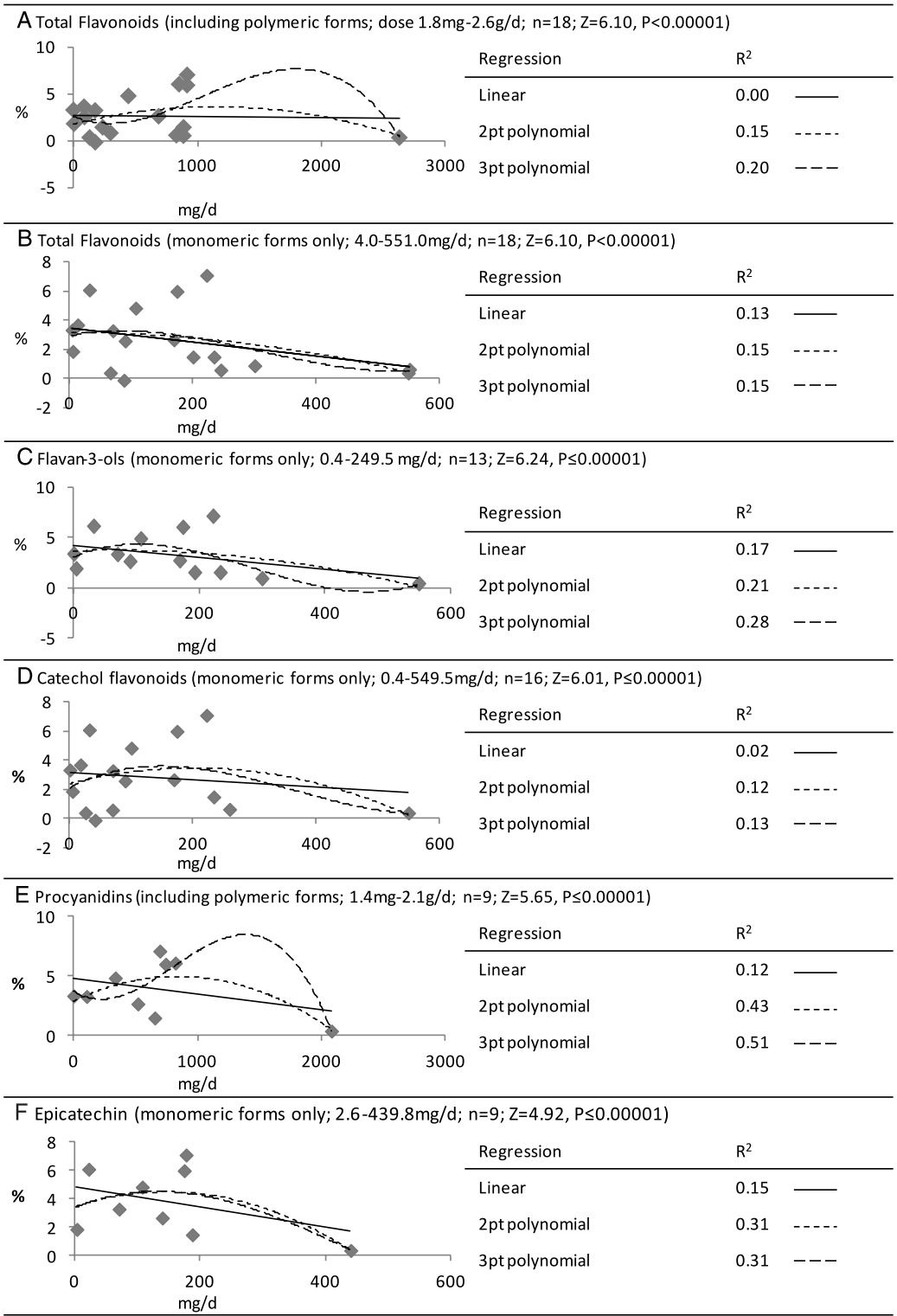


Figure 3. Acute FMD (%) dose response (mg/day). Dose-response plots represent % change in FMD response presented for random effects meta-analysis conducted using inverse variance relative to dose in mg/day. Acute, 90–150 min; FMD, flow-mediated dilation. Z scores and *p* values were derived from meta-analyses utilising fixed effects mean differences and R^2 coefficient of determination was derived from dose-response regression plots. Analysis of 'total flavonoids' represent a summation of all flavonoids within the RCTs.

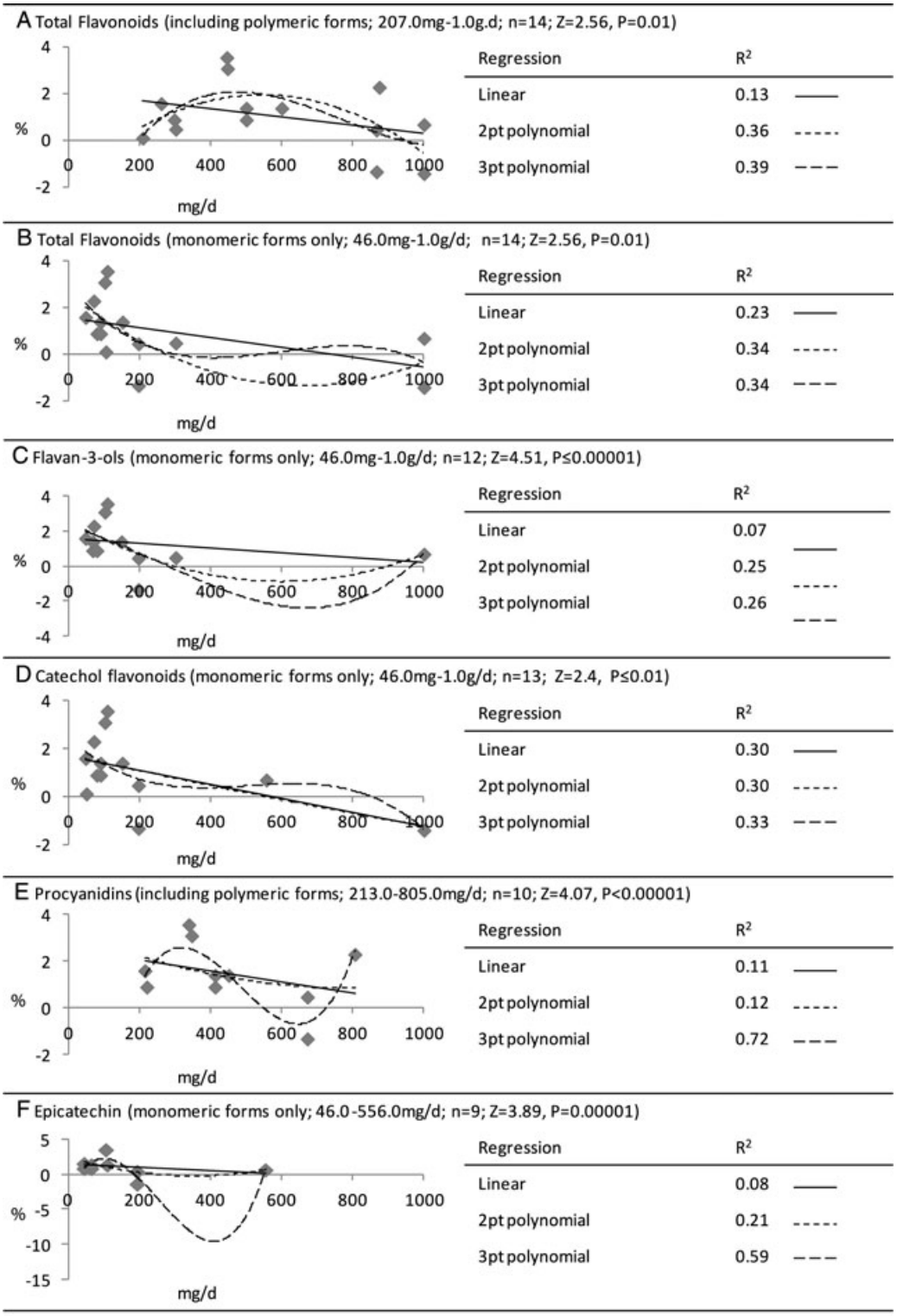


Figure 4. Chronic FMD (%) dose response (mg/day). Dose-response plots represent % change in FMD response presented for random effects meta-analysis conducted using inverse variance relative to dose in mg/day. Chronic, after a minimum of 2 weeks intervention; FMD, flow-mediated dilation. Z scores and *p* values were derived from meta-analyses utilising fixed effects mean differences and R^2 coefficient of determination was derived from dose-response regression plots. Analysis of 'total flavonoids' represent a summation of all flavonoids within the RCTs.

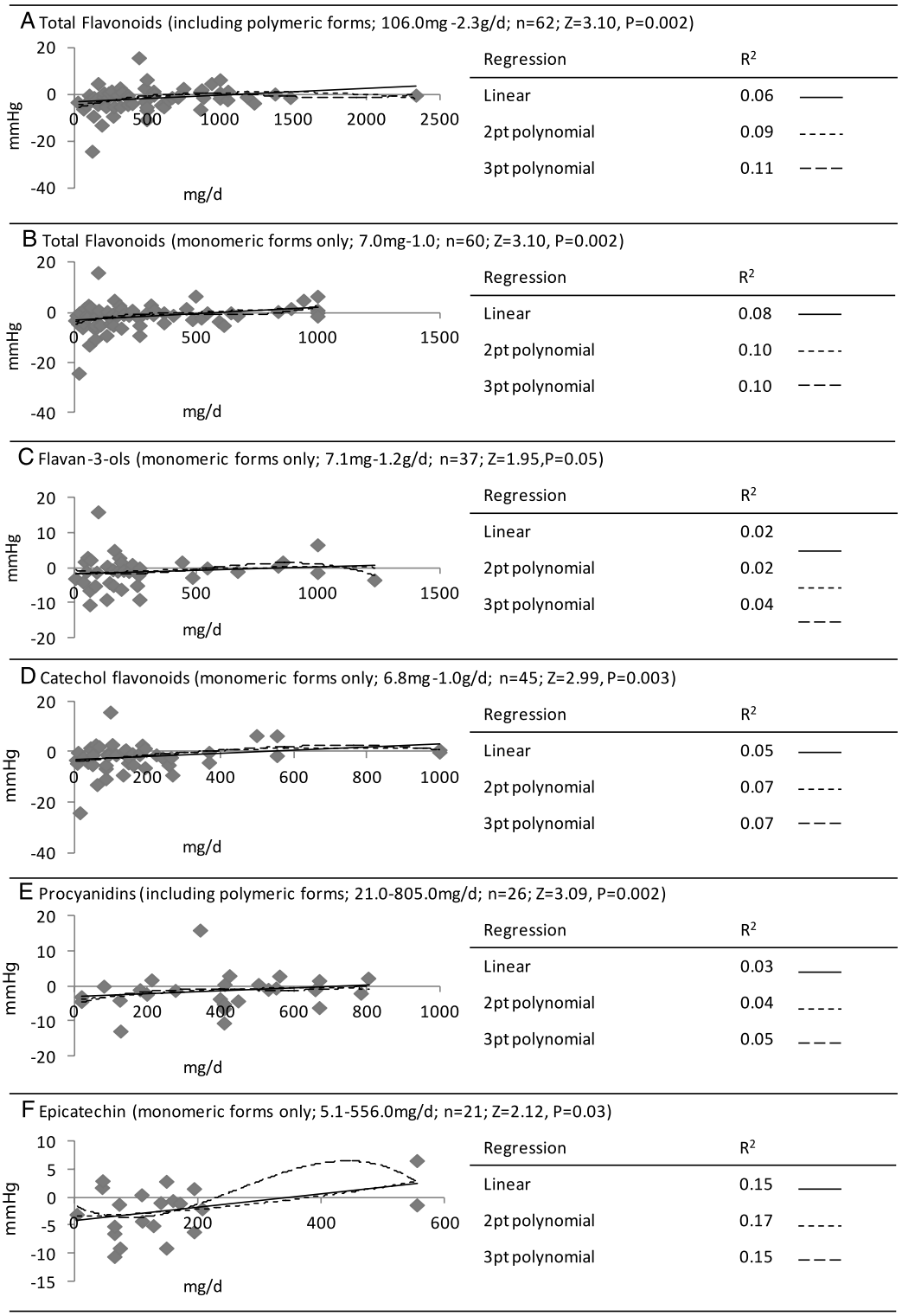


Figure 5. Chronic SBP dose response. Dose-response plots represent change in BP (mmHg), presented for random effects meta-analysis conducted using inverse variance relative to dose in mg/day. Chronic, a minimum of at least 2 weeks intervention. Z scores and *p* values were derived from meta-analyses utilising fixed effects mean differences and R^2 coefficient of determination was derived from dose-response regression plots. Analysis of 'total flavonoids' represent a summation of all flavonoids within the RCTs.

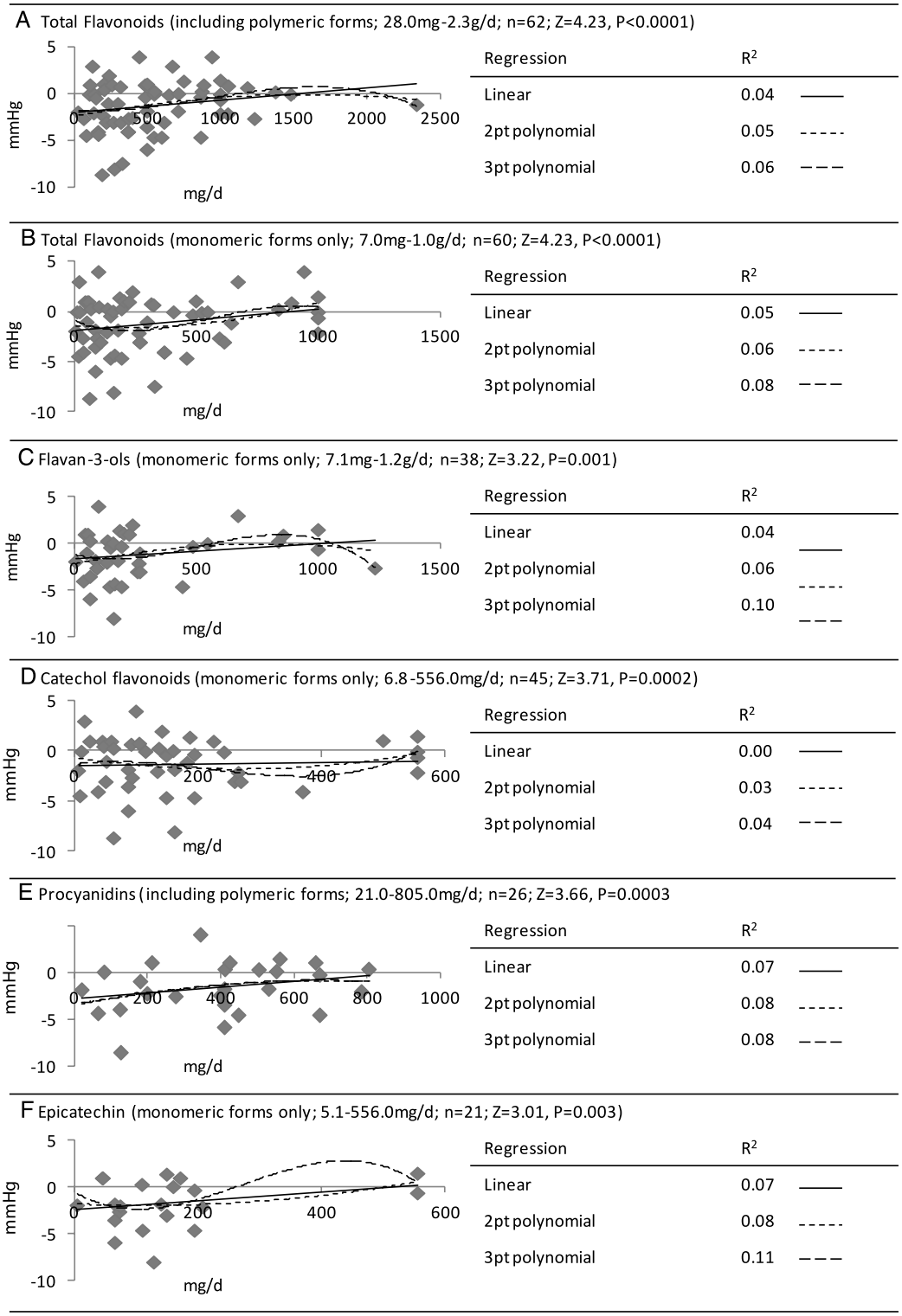


Figure 6. Chronic DBP dose-response. Dose-response plots represent change in BP (mmHg), presented for random effects meta-analysis conducted using inverse variance relative to dose in mg/day. Chronic, a minimum of at least 2 weeks intervention. Z scores and *p* values were derived from meta-analyses utilising fixed effects mean differences and R^2 coefficient of determination was derived from dose-response regression plots. Analysis of 'total flavonoids' represent a summation of all flavonoids within the RCTs.

The interpretation of many published RCTs has been challenging as a result of issues with study design. Highlighting these design limitations is essential, if progress towards understanding the bioactivity of flavonoids is to be achieved. Even though meta-analysis may not be the most appropriate tool for establishing these limitations, it does allow for the realisation of inherent 'gaps' within the literature, and in this particular instance our lack of knowledge regarding dose response. It is important to note that polynomial regression may appear to provide a better data fit in some circumstances, but high-dose studies are currently lacking from many of the available data sets; therefore, leaving the regression analyses sensitive to outliers.

In the present data set, theaflavins and thearubigins, procyanidins and condensed tannins and various gallate conjugates of catechin were each grouped together (respectively) as the complexities of their structures did not allow for their classification into single subclass. Together these compounds comprise a significant proportion of the polyphenols in flavonoid-rich foods, such as tea, chocolate and wine [9–11]. Ultimately the definitive contribution of polyphenol polymers to the bioactivity of flavonoids is difficult to ascertain as there is a lack of compositional data provided in available RCTs and flavonoid databases. Even though more extensive publically available databases will undoubtedly be available in the near future, and recent progress with composition databases has made this review possible. There are many inaccuracies with this type of post hoc analysis, particularly, because plants and therefore plant-derived foods are dynamic and the amounts of polyphenols and flavonoids will vary depending on growing conditions (sunlight, water, nutrient and pH composition of soil) and the country of origin. The only accurate way to determine the effect of flavonoid composition on a physiological response is to conduct a direct compositional analysis on the specific intervention foods, and at the times prior to and over the course of the RCT. This is a significant limitation with the majority of previous RCTs in this field. In the present systematic review, only 22% of included studies provided comprehensive flavonoid compositional analysis, while 34% provided limited, incomplete or targeted analysis and 44% provided no compositional data at all. Other general inherent weaknesses of published intervention data include low participant numbers, poor reporting of data, lack of long-duration interventions, limited dose-response analysis and lack of pharmacokinetic data. Conversely, an important conceptual limitation of the present review is the assumption that intervention studies feeding different food sources of flavonoids can be grouped together, thus suggesting food components act independently and that a pure flavonoid would act similarly to a flavonoid in a fruit or a processed food. Obviously, this is not the case and we acknowledge that this approach also comes with limitations. The utility of the present analysis was to develop novel hypotheses on the relative importance of different flavonoid subgroupings.

To our knowledge, this is the first systematic review of flavonoid interventions to focus on dose response and also the first to explore the actions of catechol B-ring flavonoids as a unique flavonoid subgrouping. In doing so, we have observed some unique associations which should help to focus future RCTs in addition to cell and animal research. In particular, it is clear that at present there is an incomplete picture of dose response across intervention studies. Importantly, our data suggest that for some classes of flavonoid there may be a maximal biological effect or dose threshold. This was particularly apparent where bioactivity did not follow a linear dose response. The indication of differential flavonoid responses across the dose range is representative of the reported bimodal bioactivity of flavonoids in some previous in vitro cellular and molecular model investigations [15–17], and has more recently been reported in a large prospective US cohort of nearly 100 00 adult men and women (of mean age >69 years) [18]. Although this observation in the present systematic review is interesting and may have significant implications, the present analysis is extrapolative in nature and designed only to form hypotheses; RCTs specifically designed to explore dose-response effects are necessary to establish if this is indeed a 'true effect'. If flavonoids do however have a dose threshold or bimodal dose response, systematic reviews combining high- and low-dose interventions together, without accounting for dose response, may overlook potentially important physiological effects of flavonoids. More importantly, if different subclasses of flavonoids have either 'inverted U-shaped', 'U-shaped' or bimodal bioactivity, this could have very significant biological/health implications. In addition, wide inter-person variability in phase II and microbial metabolites formed following ingestion of flavonoid-rich foods will likely have significant effects on the magnitude of any measured response and such variables need to also be taken into account in future studies [19].

In conclusion, this systematic review approach facilitated an exploration into the dose-response relationships of flavonoids across current RCTs, providing insight into the effects of flavonoids on biomarkers of CVD risk. The main findings indicate that there may be non-linear dose effects of flavonoid monomers and polymers and further studies are needed to establish the impact of these associations on health and disease.

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