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Flavonoid subclasses and CHD risk: a meta-analysis of prospective cohort studies

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Abstract

Epidemiological studies have shown that higher intake of flavonoid is inversely associated with CHD risk. However, which flavonoid subclass could reduce CHD risk has remained controversial. The present meta-analysis of prospective cohort studies aimed to quantitatively assess the associations between flavonoid subclasses and CHD risk. A systematic literature search was implemented from PubMed and Web of Science databases up to March 2021, and eligible studies were identified. Multivariate-adjust relative risks (RR) with corresponding 95 % CI were pooled by using a random-effects model. A restricted cubic spline regression model was performed for non-linear dose–response analysis. A total of 19 independent prospective cohort studies with 894 471 participants and 34 707 events were included. The results showed that dietary intakes of anthocyanins (RR = 0·90; 95 % CI: 0·83, 0·98), proanthocyanidins (RR = 0·78; 95 % CI: 0·65, 0·94), flavonols (RR = 0·88; 95 % CI: 0·79, 0·98), flavones (RR = 0·94; 95 % CI: 0·89, 0·99) and isoflavones (RR = 0·90; 95 % CI: 0·83, 0·98) were negatively associated with CHD risk. Dose–response analysis showed that increment of 50 mg/d anthocyanins, 100 mg/d proanthocyanidins, 25 mg/d flavonols, 5 mg/d flavones and 0·5 mg/d isoflavones were associated with 5 % reduction in CHD risk, respectively. Sensitivity and subgroup analyses were used to further support these associations. The present results indicate that dietary intakes of fruits and vegetables abundant five flavonoid subclasses, namely anthocyanins, proanthocyanidins, flavonols, flavones and isoflavones, are associated with a lower risk of CHD.

Key words: **Flavonoid subclass: CHD: Prospective cohort study: Meta-analysis**

CHD, as a typical CVD, seriously endangers health. WHO divided it into five categories, namely asymptomatic myocardial ischemia (occult CHD), angina pectoris, myocardial infarction, ischemic heart disease and sudden death[1]. According to epidemiological studies, the incidence of CHD has remained stable while the mortality rate has decreased, indicating that the prevention strategy of CHD is supposed to improve[2]. The pathogenesis of CHD is influenced by genetic and environmental factors, thereby several studies put forward the idea of improving dietary and behavioural habits to prevent and ameliorate CHD[3].

Flavonoid, as a type of polyphenol, composes of two benzene rings with oxygen-containing heterocyclic rings. Substantial evidence has reported that phytochemicals, abundant in fruits and vegetables, could improve and prevent various chronic diseases, such as CVD, stroke and several cancers[4–6]. Varieties of flavonoid subclasses are formed due to different permutations, molecular compositions and linking compounds. Generally speaking, flavonoid subclasses include flavones, flavonols, flavanones, anthocyanins flavan-3-ols, isoflavones, proanthocyanidins, and so on[7]. To date, a dose–response meta-analysis has demonstrated that higher intakes of flavonoids are associated with reducing CVD risk, possible through anti-inflammatory and antioxidant mechanisms[8]. Notably, due to various molecular structures and biological bioavailability, different flavonoid subclasses might have various effects on CHD[9,10]. A major controversial has persisted over which flavonoid subtypes have ameliorative effects on CHD. Therefore, we proposed a hypothesis that certain subclasses of flavonoids might be associated with reducing CHD risk, while other subclasses had no significant impact or performed a stimulating effect.

In the present study, we conducted a systematic review and meta-analysis of nineteen prospective cohort studies and aimed to address the associations of flavonoid subclasses with CHD risk, including flavonols, flavanones, flavones, anthocyanins, proanthocyanidins, isoflavones and flavan-3-ols[11–29]. Furthermore, dose–response analyses were conducted to

Abbreviations: RR, relative risk.

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quantitatively explore the associations of flavonoid subclasses with CHD risk.

Methods

Search strategy and inclusive criteria

A systematic search was conducted with PubMed and Web of Science databases up to March 2021. Flavonol, flavanone, flavone, anthocyanin, quercetin, kaempferol, myricetin, proanthocyanidin, isoflavone, flavan-3-ol and flavonoid were combined with CHD as search term. There was no restriction on the language of publication. The exposures of interest were flavonoid subclasses, and the outcome of interest was the incidence of CHD. Inclusion criterion for publication was prospective cohort study. Additionally, manual search was performed to examine relevant systematic reviews, meta-analyses and original publications. These processes were repeated until we found no more relevant publications.

Data extraction and quality assessment

The basic characteristics of the inclusive studies were extracted: first author's surname, publication year, number of participants and cases, characteristics of the participants and age at baseline, duration of follow-up, type and dose of exposure, measurement methods of exposure and outcome, and adjustment for potential confounding factors. The multivariate-adjusted relative risks (RR) with corresponding 95 % CI were extracted for data synthesis. Discrepancies with the inclusion of studies and interpretation of data were resolved by panel discussion. In addition, the Newcastle–Ottawa Scale quality assessment was adopted for evaluating the quality of the inclusion publications[30]. The scoring system consisted of eight aspects: four for selection, one for comparability and three for assessment of outcome. The highest score was nine stars, with 0–3, 4–6 and 7–9 stars were indicative of low, medium and high quality, respectively.

Statistical analysis

For the inclusive studies, the Cox proportional hazard model was implemented to assess the relationships between flavonoid subclasses intake and CHD risk. Multivariate-adjust RR with corresponding 95 % CI for the highest *v.* the lowest intake category were pooled by using a random-effects model. Furthermore, dose–response analyses were conducted to quantitatively explore the relationships between flavonoid subclasses and CHD risk. The midpoint of the upper and lower bounds was regarded as the dose of quantile if the inclusive study only provided the range. If the highest quantile was open-ended, its dose was defined as 1·2-fold the highest boundary. A two-stage random effects dose-response analysis was performed to evaluate these associations. To estimate whether there was a non-linear (curvilinear) relationship between flavonoid subclass intake and CHD risk, we used a restricted cubic spline model with three knots at fixed percentiles of the flavonoid subclasses' distribution (25 %, 50 % and 75 %). A *P*-value of curvilinear was calculated by assuming that the regression coefficient of the second spline was equal to 0[31]. In the presence of substantial linear trend (*P*-value for curvilinear > 0·05), a linear dose-response meta-analysis was implemented to evaluate the relationships between flavonoid subclasses increment and CHD risk.

The I^2 statistics described the between-study heterogeneity with 25·0 %, 50·0 % and 75·0 % as cut-off points to define low, medium and high heterogeneity, respectively. In the case of substantial heterogeneity, subgroup and univariate meta-regression analyses were carried out based on following information, including region, duration of follow-up and mean age of participants. Sensitivity analysis was performed to assess whether one study exerted substantial effect on the summary estimate. Begg's rank correlation test was adopted to evaluate publication bias (significant level at $P < 0{\cdot}1$)[32]. Statistical analysis was performed by using STATA 15.0 for windows (StataCorp).

Results

Study selection

The search process is shown in Fig. 1. After removing 766 repeated references, 3170 unique articles were searched from PubMed and Web of Science databases. After screening titles and abstracts, 3123 citations were deleted, such as reviews, case–control studies and animal models, leaving 47 articles for full-text search. Among them, twenty-eight articles were excluded due to they did not meet the criteria, such as useable data, basic information and exposure of interest were not provided. Finally, nineteen prospective cohort studies were included for data analysis[11–29].

Study characteristics

The characteristics of the study are listed in Table 1. All the studies were published between 1996 and 2021. Thirteen studies obtained exposure information through FFQ[11,13,14,16,18,20–24,26–28]. Among the studies reviewed, nine studies consisted of both men and women[12,13,19–21,23,25,28,29], three consisted of only men[15,17,24] and seven consisted of only women[11,14,16,18,22,26,27]. With regard to geographical region, nine studies were conducted in the USA[11,16–18,20–22,24,28], eight studies in Europe[12–15,19,25,26,29] and two studies in Australia and Japan[23,27], respectively. Based on Newcastle–Ottawa Scale quality assessment, one study was classified as medium quality[19], and the remaining eighteen studies were regarded as high quality[11–18,20–29] (online Supplementary Table 1).

Flavonoid subclasses and CHD risk

The meta-analysis results of flavonoid subclasses with CHD risk are shown in Table 2. Eight prospective cohort studies were integrated to describe the association of anthocyanin intake with CHD risk[13,18,20–22,24,25,27], and the summary estimate showed that higher anthocyanin intake was associated with 10 % (95 % CI: 0·83, 0·98) reduction in risk of CHD, with moderate heterogeneity ($I^2 = 57{\cdot}4$ %, $P = 0{\cdot}022$) (Fig. 2). The non-curvilinear association was observed between anthocyanin intake and CHD risk by using the restricted cubic spline model (*P*-value for curvilinear = 0·299) (online Supplementary Fig. 1).

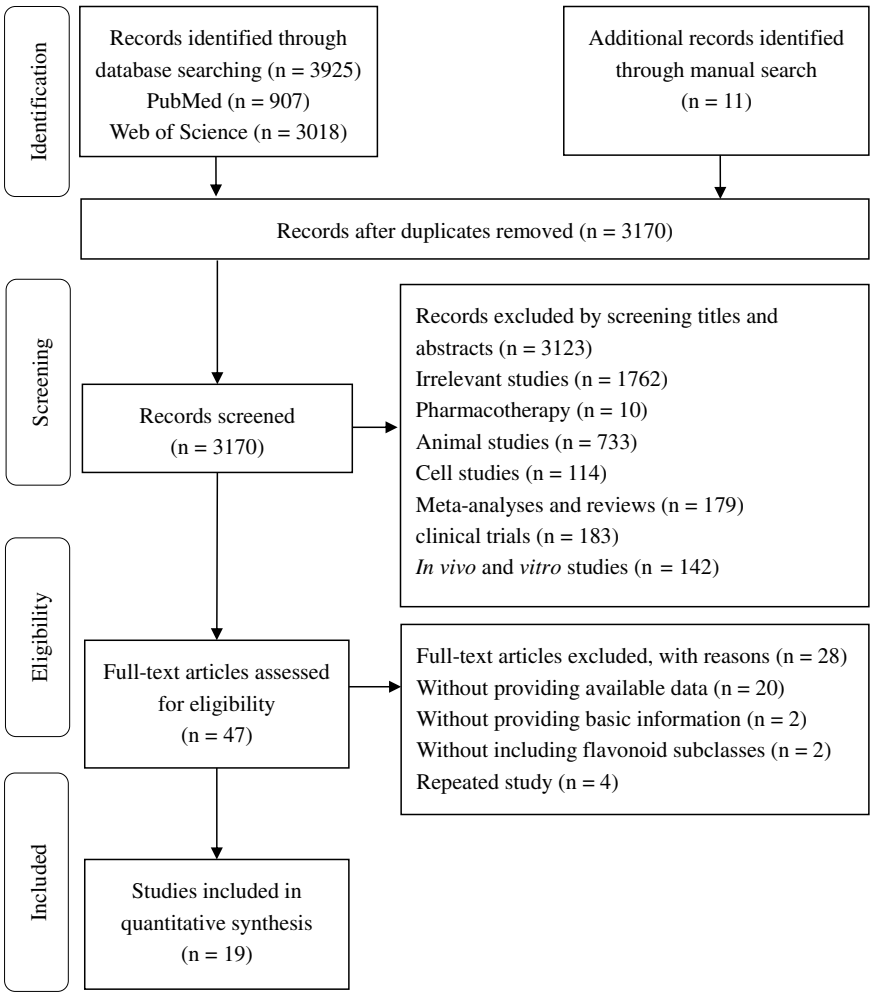

Fig. 1. The process of study selection.

Dose-response analysis showed that 50 mg per day increment of anthocyanin intake was associated with 5 % (95 % CI: 0·92, 0·99; $P_{\text{for trend}} = 0{\cdot}030$) reduction in CHD risk.

Four prospective cohort studies reported an association between proanthocyanidin intake and CHD risk[20,22,25,27], and the pooled RR was 0·78 (95 % CI: 0·65, 0·94), with non-significant between-study heterogeneity ($I^2 = 35{\cdot}0\,\%$, $P = 0{\cdot}202$) (Fig. 3). There was no evidence of a non-linear association between proanthocyanidin intake and CHD risk (*P*-value for curvilinear = 0·490) (online Supplementary Fig. 2). The linear analysis described that the pooled RR of CHD risk for a 100 mg per day increment in proanthocyanidins intake was 0·95 (95 % CI: 0·93, 0·99; $P_{\text{for trend}} = 0{\cdot}003$).

In the meta-analysis of flavonol intake and CHD risk, eight prospective cohort studies were included[13,14,18,20–22,25,27]. The summary effect showed that higher intakes of flavonols were associated with 12 % (95 % CI: 0·79, 0·98) reduction in risk of CHD, with no between-study heterogeneity was observed ($I^2 = 42{\cdot}8\,\%$, $P = 0{\cdot}093$) (Fig. 4). Although non-curvilinear association was observed between flavonol intake and CHD risk (*P*-value for curvilinear = 0·945) (online Supplementary Fig. 3), linear trend was significant that 25 mg per day increment of flavonols associated with 5 % (95 % CI: 0·95, 0·97, $P_{\text{for trend}} < 0{\cdot}001$) reduction in risk of CHD.

Seven prospective cohort studies reported the association between flavone intake and CHD risk[13,18,20–22,25,27]. The pooled effect showed that intakes of flavones were associated with 6 % (95 % CI: 0·89, 0·99) reduced risk of CHD, with non-significant between-study heterogeneity ($I^2 = 0{\cdot}0\,\%$, $P = 0{\cdot}628$) (Fig. 5). The curvilinear relationship of flavone intake associated with CHD risk was not observed (*P*-value for curvilinear = 0·833) (online Supplementary Fig. 4). Linear analysis showed that the pooled RR of CHD risk for a 5 mg per day increment in flavone intake was 0·95 (95 % CI: 0·93, 0·97; $P_{\text{for trend}} < 0{\cdot}001$). In addition, an independent cohort study showed that intakes of flavones and flavonols could reduce the risk of CHD[15].

The association between isoflavone intake and CHD risk was determined by six prospective cohort studies[22,23,25–28]. The summary estimate indicated that the pooled RR for CHD was 0·90 (95 % CI: 0·83, 0·98) decreased risk of CHD, and no between-study heterogeneity was performed ($I^2 = 10{\cdot}3\,\%$, $P = 0{\cdot}350$) (Fig. 6). There was no evidence of a curvilinear relationship between isoflavone intake and CHD risk (*P*-value for curvilinear = 0·428) (online Supplementary Fig. 5). The result

Table 1. Characteristics of included studies regarding flavonoids intake and CHD risk

First author	Publication year and region	Age (y) (sex)	Subjects (cases)		Follow-up period (y)	Exposure measure	Outcome measure	Exposure	RR	95 % CI	Covariates adjusted
Hertog[14]	1996, Caerphilly	52·1 (F)	1900	131	14·0	FFQ	Death certificates	Flavonols Quercetin	1·10 1·10	0·60, 1·80 0·70, 1·71	Age, smoking, baseline evidence of ischemic heart disease, social class, BMI, systolic blood pressure, serum total cholesterol and intakes of total energy, alcohol, fat, vitamin C, vitamin E and β-carotene.
Rimm[17]	1996, USA	55·2 (M)	34 789	496	6·0	131-item questionnaires	Report of next of kin, co-workers and postal authorities	Quercetin Kaempferol Myricetin	1·14 0·99 0·89	0·86, 1·51 0·75, 1·31 0·67, 1·19	Age, BMI, smoking, diabetes, intake of vitamin E, intake of alcohol, hypertension, high cholesterol level, family history of CHD and profession.
Yochum[11]	1999,USA	61·6 (F)	34 492	438	10·0	FFQ	The state health registry of Iowa collects information	Quercetin Kaempferol Myricetin	0·74 0·79 0·86	0·52, 1·06 0·57, 1·09 0·63, 1·16	Age, total energy intake, BMI squared, waist-to-hip ratio, high blood pressure, diabetes, oestrogen replacement therapy, alcohol intake, education, marital status, pack-years of smoking and physical activity.
Hirvonen[15]	2001, Finland	56·8 (M)	25 372	1937	6·1	Diet history questionnaire, the food consumption data	Hospital record, the register of causes of death	Flavonols and Flavones	0·77	0·64, 0·93	Age, supplementation group, systolic and diastolic blood pressure, serum total cholesterol, serum high-density lipoprotein cholesterol, BMI, smoking years, number of cigarettes smoked daily, history of diabetes mellitus or CHD, marital status, educational level and physical activity.
Knekt[12]	2002, Finland	54·0 (F/M)	10 054	681	28·0	A dietary history method, questionnaire	Finland statistics data	Quercetin Kaempferol Myricetin	0·79 0·82 1·14	0·63, 0·99 0·66, 1·02 0·92, 1·41	Age, sex, geographic area, occupation, blood pressure, smoking, serum cholesterol, BMI and diabetes.
Schouw[26]	2004, European	57·1 (F)	16 165	372	3·1	FFQ	The Dutch Center for Health Care Information	Isoflavones	0·94	0·68, 1·30	Age, BMI, smoking, physical activity, diabetes mellitus, hypertension, hypercholesterolemia, HRT use, energy intake, animal protein intake, monounsaturated fat intake, fibre intake, alcohol intake, fruit intake and vegetable intake.
Marniemi[19]	2005, Finland	82·0 (F/M)	755	130	10·0	Dietary history interview	The national register of causes of death	Quercetin Kaempferol Myricetin	0·69 0·85 1·04	0·44, 1·09 0·56, 1·28 0·68, 1·60	Age, sex, smoking, functional capacity and weight-adjusted energy intake.
Lin[16]	2007, USA	56·3 (F)	66 360	1262	12·0	FFQ	The US postal service or ascertain from the national death record	Quercetin Kaempferol Myricetin	1·01 0·99 1·08	0·85, 1·21 0·83, 1·18 0·90, 1·29	Age, current smoking, parental history of MI before age 60 years, history of hypertension, hypercholesterolemia, diabetes, menopausal status, postmenopausal hormone use, use of aspirin, use of multivitamin and vitamin E supplements, BMI, physical activity, alcohol consumption, and total energy intake.
Mink[22]	2007, USA	61·8 (F)	34 489	1329	16·0	FFQ	Follow-up questionnaires	Anthocyanins Flavanones Flavones Flavonols Isoflavones Flavan-3-ols Proanthocyanidins	0·88 0·78 0·95 0·95 1·00 1·02 0·91	0·78, 0·99 0·65, 0·94 0·79, 1·15 0·79, 1·14 0·84, 1·20 0·86, 1·21 0·76, 1·09	Age, energy intake, marital status, education, blood pressure, diabetes, BMI, waist-to-hip ratio, physical activity, smoking and oestrogen use.
Kokubo[23]	2007, Japanese	40·0–59·0 (F/M)	40 462	308	12·0	FFQ	The criteria of the MONICA project	Isoflavones	M 0·77 F 0·37	0·47, 1·25 0·14, 0·98	Age, sex, smoking, alcohol use, BMI, history of hypertension or diabetes mellitus, medication use for hypercholesterolemia, education level, sports, dietary intake of fruits, vegetables, fish, salt, and energy, menopausal status for women and PHC.
Cassidy[18]	2013, USA	36·9 (F)	93 600	405	18·0	FFQ	Medical records, autopsy results, death certificate	Flavonols Flavones Flavanones Flavan-3-ols Anthocyanins	0·79 1·00 0·91 0·82 0·68	0·58, 1·08 0·72, 1·39 0·66, 1·26 0·61, 1·11 0·49, 0·95	Age, physical activity, smoking, BMI, alcohol, energy, menopausal status, PMH use, aspirin use, oral contraceptive use, family history of MI, cereal fibre, SFA, *trans*-fatty acids, PUFA, MUFA, caffeine.
Ivey[27]	2013, Australian	81·0 (F)	1063	64	5·0	FFQ and beverage intake data.	The Western Australian Data Linkage System for each of the study participants	Flavonols Flavan-3-ols Proanthocyanidins Flavones Flavanones Anthocyanins Isoflavones	0·27 0·34 0·62 0·56 0·55 0·67 0·87	0·13, 0·58 0·17, 0·69 0·32, 1·20 0·28, 1·10 0·28, 1·09 0·33, 1·35 0·46, 1·64	Age, previous CVD, previous diabetes, energy expended in physical activity and history of smoking.
Jacques[21]	2015, USA	54·2 (F/M)	2880	261	14·9	FFQ	Hospitalisation records, physician office visit records, autopsy results, Framingham heart study records	Flavonols Flavones Flavanones Flavan-3-ols Anthocyanins	0·90 0·87 0·96 1·00 0·96	0·73, 1·11 0·71, 1·07 0·87, 1·06 0·88, 1·13 0·85, 1·08	Age, sex, current smoking status, BMI and total energy intake.
Vogiatzoglou[29]	2015, European	59·0 (M) 58·0 (F) 64·0 (PF)	11 252 13 633 8176	2335 1325 1157	11·1	7-day food diaries	ENCORE	Flavan-3-ols	M 0·88 F 0·96 PF 0·84	0·77, 1·01 0·79, 1·16 0·69, 1·02	BMI, energy intake, systolic blood pressure, plasma cholesterol, plasma vitamin C, intake of fibre, K, Na, fat, saturated fat, alcohol, and the following categorical variables-physical activity, smoking status, education, self-reported history of stroke, MI and diabetes at baseline, family history of myocardial infarction, use of antihypertensive and lipid-lowering drugs; additional in women use of hormone replacement therapy.

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Table 1. (*Continued*)

First author	Publication year and region	Age (y) (sex)	Subjects (cases)	Follow-up period (y)	Exposure measure	Outcome measure	Exposure	RR	95 % CI	Covariates adjusted
Goetz[20]	2016, USA	64·2 (F/M)	30 239	4·1–7·9	A block98 FFQ	Telephone surveillance, hospitalisations report, medical record retrieval, report of next of kin	Anthocyanins	0·73	0·55, 0·96	Age, energy and sex, race, region of residence, educational attainment, household income, exercise, smoking status, percentage of energies from sweets, fibre, *trans*-fat, and *n*-3 fatty acids, BMI, aspirin use, history of or use of medications for hypertension, diabetes or hyperlipidemia, CHD, reasons for geographic and racial disparities in stroke.
							Flavanones	1·03	0·80, 1·33	
							Flavonols	0·98	0·75, 1·28	
							Flavan-3-ols	1·05	0·82, 1·34	
							Flavones	0·97	0·73, 1·28	
							Proanthocyanidins	0·66	0·51, 0·85	
Cassidy[24]	2016, USA	53·6 (M)	43 880	24·0	FFQ	Questionnaire, medical records, autopsy results	Flavanones	0·98	0·88, 1·09	Age, physical activity, smoking, BMI, alcohol, energy, cereal fibre, folate, SFA, PUFA, *trans*-fatty acids, marital status, hypertension, hypercholesterolemia and family history of MI.
							Anthocyanins	0·97	0·87, 1·08	
Adriouch[25]	2018, French	49·3 (F/M)	81 458	4·9	Questionnaires and 24-h dietary records	Health status questionnaire, check-up questionnaire	Anthocyanins	0·71	0·51, 0·98	Age, BMI, physical activity, smoking status, numbers of dietary records, alcohol intake, energy intake, family history of CVD, educational level and season of completion of 24-h dietary records.
							Flavanones	1·21	0·91, 1·60	
							Flavones	1·13	0·83, 1·53	
							Flavonols	0·83	0·62, 1·12	
							Isoflavones	1·04	0·78, 1·38	
							Proanthocyanidins	0·80	0·58, 1·10	
Dalgaard[13]	2019, Danish	55·0–65·0 (F/M)	53 552	21·0	FFQ	The Danish National Patient Register	Flavonols	0·87	0·82, 0·92	Age, sex, BMI, smoking status, physical activity, alcohol intake, and social economic status, intakes of fish, red meat, processed food, PUFA, MUFA and SFA.
							Flavones	0·94	0·88, 1·00	
							Flavanones	0·96	0·91, 1·02	
							Anthocyanins	1·01	0·95, 1·08	
Ma[28]	2020, USA	30·0–55·0 (F)	121 700	28·0	FFQ	The follow-up questionnaires, hospital records, autopsy reports, medical records	Isoflavones	0·87	0·81, 0·94	Age, ethnicity, self-rated socio-economic status, partner's education, smoking status, alcohol intake, physical activity, multivitamin use, aspirin use, history of hypertension and hypercholesterolemia, family history of MI, menopausal status and postmenopausal hormone use, oral contraceptive use, BMI, and total energy intake, modified alternative Health Eating Index score.
		25·0–42·0 (F)	116 671	22·0						
		40·0–75·0 (M)	51 529	26·0						

y, year; RR, relative risk; F, female; M, male; HRT, hormone replacement therapy; MI, myocardial infarction; MONICA, monitoring of trends and determinants in cardiovascular disease; PHC, public health centre-based; PMH, previous medical history; ENCORE, East Norfolk commission record; PF, postmenopausal female.

Table 2. The meta-analysis results of flavonoid subclasses with CHD risk

Exposure	RR	95 % CI	I^2 (%)	*P*
Anthocyanin	0·90	0·83, 0·98	57·4	0·022
Proanthocyanidin	0·78	0·65, 0·94	35·0	0·202
Flavonol	0·88	0·79, 0·98	42·8	0·093
Flavone	0·94	0·89, 0·99	0·0	0·628
Isoflavone	0·90	0·83, 0·98	10·3	0·350
Flavanone	0·95	0·89, 1·02	33·7	0·159
Flavan-3-ol	0·92	0·84, 1·02	48·3	0·060
Kaempferol	0·91	0·91, 1·01	0·0	0·562
Myricetin	1·03	0·92, 1·15	0·0	0·494
Quercetin	0·91	0·78, 1·07	41·5	0·129

RR, relative risk; *P*, value for heterogeneity within subgroup.

showed that the linear relationship was obvious between isoflavone intake and CHD risk ($P_{\text{for trend}} < 0{\cdot}001$) and revealed that 0·5 mg per day increment of isoflavone intake was associated with 5 % (95 % CI: 0·94, 0·97) reduction in CHD risk.

In the meta-analysis of flavanone intake and CHD risk, eight prospective cohort studies were included[13,18,20–22,24,25,27], and in that of flavan-3-ol intake and CHD risk, six studies were included[18,20–22,27,29]. The pooled RR for CHD risk were 0·95 (95 % CI: 0·89, 1·02) for flavanones and 0·92 (95 % CI: 0·84, 1·02) for flavan-3-ols (online Supplementary Fig. 6 and 7). There was no evidence of between-study heterogeneity of flavanone ($I^2 = 33{\cdot}7$ %, $P = 0{\cdot}159$) and flavan-3-ol ($I^2 = 48{\cdot}3$ %, $P = 0{\cdot}060$) with CHD risk.

Individual flavonols and CHD risk

Six prospective cohort studies reported an association between quercetin intake and CHD risk[11,12,14,16,17,19]. Five prospective cohort studies reported the associations of kaempferol and myricetin intake with CHD risk[11,12,16,17,19]. The pooled effect showed that intakes of quercetin (RR = 0·91; 95 % CI: 0·78, 1·07), kaempferol (RR = 0·91; 95 % CI: 0·81, 1·01) and myricetin (RR = 1·03; 95 % CI: 0·92, 1·15) were not significantly associated with reduced CHD risk, respectively (online Supplementary Fig. 8–10). Moreover, there were no between-study heterogeneity among quercetin ($I^2 = 41{\cdot}5$ %, $P = 0{\cdot}129$), kaempferol ($I^2 = 0{\cdot}0$ %, $P = 0{\cdot}562$) and myricetin ($I^2 = 0{\cdot}0$ %, $P = 0{\cdot}494$), respectively (Table 2).

Publication bias and sensitivity analysis

No publication biases were observed among the studies of flavonols ($P = 0{\cdot}538$), flavones ($P = 0{\cdot}764$), flavanones ($P = 0{\cdot}711$), flavan-3-ols ($P = 0{\cdot}108$), proanthocyanidins ($P = 0{\cdot}734$), isoflavones ($P = 0{\cdot}230$), quercetin ($P = 0{\cdot}452$), kaempferol ($P = 0{\cdot}462$) and myricetin ($P = 0{\cdot}462$) by using Begg's rank correlation test. However, there was markedly publication bias with respect to anthocyanins ($P = 0{\cdot}019$). Thus, the trimming and filling method was implemented to deal with publication bias and obtain the funnel plot (online Supplementary Fig. 11). In the sensitivity analysis, each study was excluded sequentially and recalculated the pooled RR. The results reported that the remaining data were not substantially driven by deletion of each study (online Supplementary Fig. 12–22).

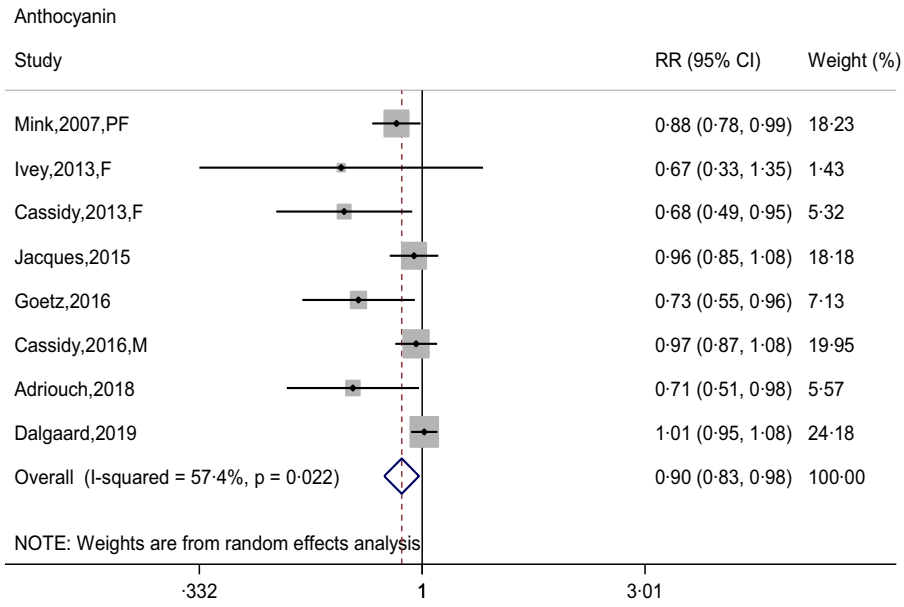

Fig. 2. Forest plot to quantify the association between anthocyanins and CHD risk. The summary RR was calculated by using a random-effects model for the highest *v.* lowest category. RR, relative risk; PF, postmenopausal female; F, female; M, male.

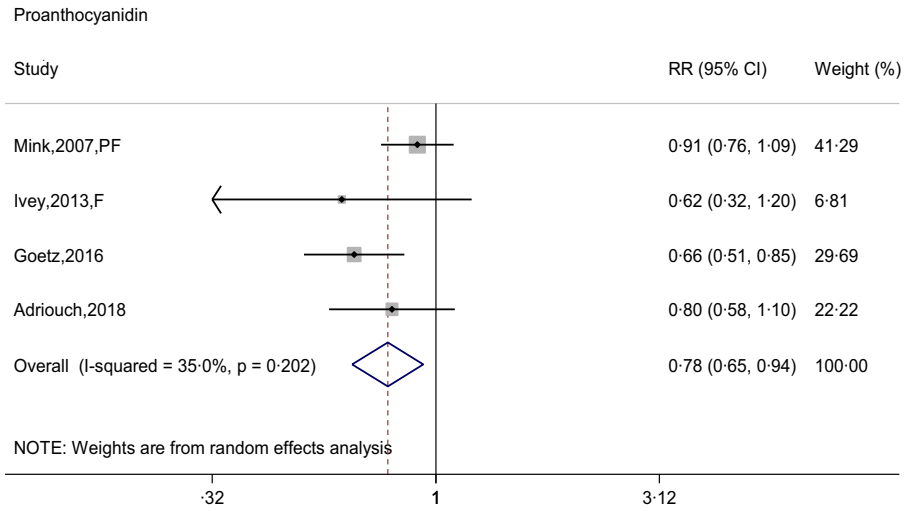

Fig. 3. Forest plot to quantify the association between proanthocyanidins and CHD risk. The summary RR was calculated by using a random-effects model for the highest *v.* lowest category. RR, relative risk; PF, postmenopausal female; F, female.

Subgroup analysis

Due to significant between-study heterogeneity, subgroup analysis was conducted according to mean age, region and duration of follow-up. The pooled effect showed that intakes of anthocyanins were inversely associated with the risk of CHD in America (pooled RR = 0·89; 95 % CI: 0·81, 0·99) (Table 3). And the inverse relationship was not significant in other regions.

Discussion

The present meta-analysis of prospective cohort studies was the first to systematically analyse the relationships between flavonoid subgroups and CHD risk. The present meta-analysis consisted of 19 prospective cohort studies, including 894 471 participants and 34 707 CHD events. The summary results demonstrated that higher intakes of anthocyanins, proanthocyanidins, flavonols, isoflavones and flavones were inversely associated with CHD risk. Although the curvilinear relationships of these flavonoid subclasses with CHD risk were not significant, linear analysis results showed that increasement of 50 mg per day anthocyanins, 100 mg per day proanthocyanidins, 25 mg per day flavonols, 5 mg per day flavones and 0·5 mg per day isoflavones were associated with 5 % reduction in CHD risk, respectively.

The main pathogenic factors of CHD include genetic factors, unbalanced dietary habits, hypertension, diabetes and hyperlipidemia[33–35]. The main pathogenesis of CHD includes: (1) due to the lack of negative feedback regulation, phagocytes phagocytose highly oxidised LDL and convert to macrophage

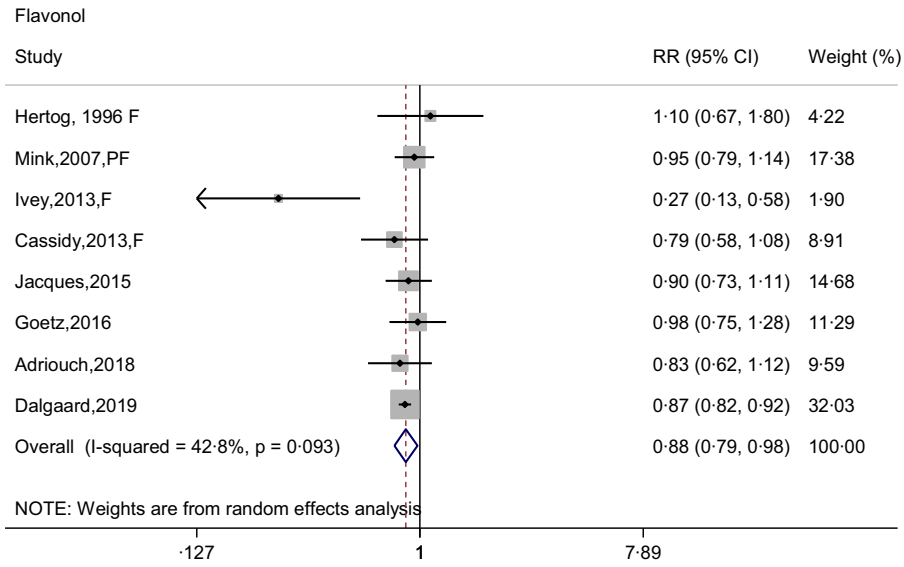

Fig. 4. Forest plot to quantify the association between flavonols and CHD risk. The summary RR was calculated by using a random-effects model for the highest *v.* lowest category. RR, relative risk; F, female; PF, postmenopausal female.

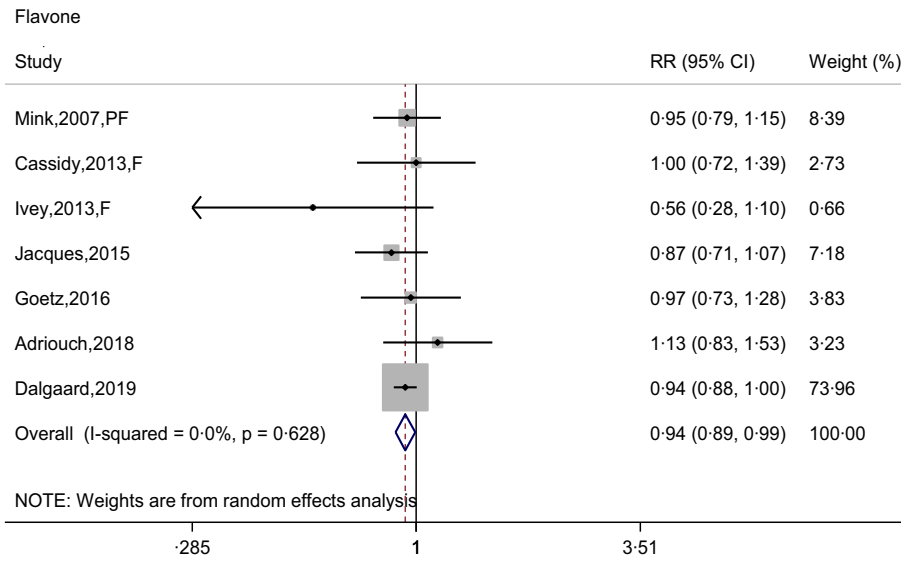

Fig. 5. Forest plot to quantify the association between flavones and CHD risk. The summary RR was calculated by using a random-effects model for the highest *v.* lowest category. RR, relative riskl; PF, postmenopausal female; F, female.

foam cells, causing a large amount of lipid accumulation to form plaques[36]. (2) Macrophages are stimulated to produce inflammation-promoting factors, which could promote the inflammatory response of plaques, thereby causing the proliferation and migration of vascular fibroblasts and smooth muscle cells[37]. (3) Substances such as oxidised LDL and free radicals released by the accumulation of foam cells could aggravate the damage of vascular endothelial cells and promote the progression of CHD[38].

It was compelling that dietary intakes of fruits and vegetables were associated with a reduced risk of CHD[39,40]. Flavonoids are exited most abundantly in fruits, vegetables, cocoa, nuts and soyabean. Multiple studies reported that higher intakes of anthocyanins and proanthocyanidins were significantly associated with reduced risk of CVD and all-cause mortality[41,42]. Three prospective cohort studies indicated that higher intakes of isoflavones significantly reduced the incidence of CHD in women, especially postmenopausal female and younger women[28]. Based on these studies, we proposed a hypothesis that only several flavonoid subgroups in food might be beneficial for reducing CHD risk.

The basic structure of flavonoid consists of C6-C3-C6 rings with different substitution patterns to produce a series of subclass compounds[43]. The numbers and types of substituent groups, such as hydroxyl groups, on the C6-C3-C6 ring are the major factors to affect the antioxidant activities[44]. In addition, non-structural factors, such as hydrophobicity and polymerisation degree, play a vital role in affecting flavonoid function.

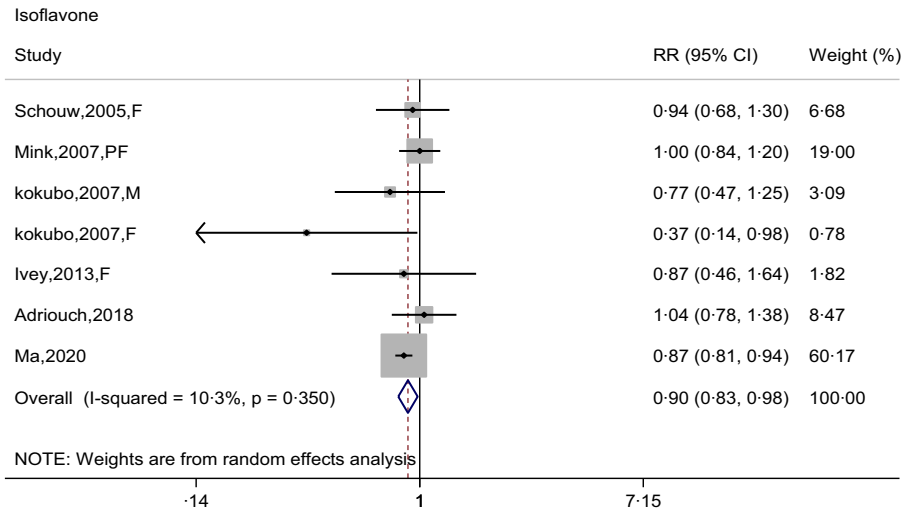

Fig. 6. Forest plot to quantify the association between isoflavones and CHD risk. The summary RR was calculated by using a random-effects model for the highest *v.* lowest category. RR, relative riskl; F, female; PF, postmenopausal female; M, male.

Table 3. Subgroup analysis of anthocyanins associated with CHD

	Anthocyanins					
				Heterogeneity		
Factors stratified	*n*	Pooled effect	95 % CI	I^2 (%)	P^a	P^b
Mean age (years)						0·912
≤ 55	4	0·89	0·78, 1·02	56·2	0·077	
> 55	4	0·90	0·77, 1·02	67·0	0·028	
Region						0·970
Oceania	1	0·67	0·33, 1·35	0·0	<0·001	
America	5	0·89	0·81, 0·99	48·5	0·100	
Europe	2	0·88	0·63,1·23	76·8	0·038	
Duration of follow-up (years)						0·371
≤ 15	4	0·82	0·67, 1·00	49·6	0·114	
> 15	4	0·94	0·85, 1·03	64·0	0·039	

No., number of included studies; P^a, value for heterogeneity within subgroup; P^b, value for heterogeneity between subgroups with meta-regression analysis; y, year.

Thus, it was indispensable and significant to clarify which flavonoid subclass was inversely associated with CHD risk. Based on this, the possible mechanisms of flavonoids subgroups in reducing the risk of CHD were put forward.

The overall possible mechanisms of five flavonoid subclasses contributed to reducing CHD risk were summarised as follows (Fig. 7): (1) inhibiting a variety of oxidases, such as xanthine oxidase and lipoxygenase, thereby inhibiting the production of peroxide free radicals[45]. Scavenging free radicals and peroxides, such as superoxide anions and reactive oxygen species, by activating the natural antioxidant system and the role of antioxidant enzymes such as superoxide dismutase and GSH peroxidase (GSH-Px), and increasing the types and role of antioxidant factors such as vitamin E, vitamin C and GSH[46–48]. (2) Reducing the gene expression of leucotriene receptor and cyclo-oxygenase-2 (COX-2), and inhibiting LDL oxidation and macrophage foam cells production to reduce inflammation[49–51]. (3) Activating the protein kinase (Akt) and phosphoinositide 3 kinase (PI3k) pathway of vascular endothelial cells, causing nitric oxide synthase phosphorylation to produce NO, thus achieving the effect of dilating blood vessels[52]. Based on the above mechanisms, flavonoid subclasses could protect against CHD risk by improving cardiovascular system, dilating blood vessels, enhancing antioxidant and inhabiting inflammation.

This meta-analysis had the following advantages to be highlighted. First, nineteen independent prospective cohort studies with a large sample-size were included to investigate the associations between flavonoid subclasses and CHD risk, thus it had a strong statistical power to detect these associations. Second, the majority of the inclusive studies were high-quality studies, except one moderate-quality study. Third, sensitivity analyses demonstrated that the summary estimates were not driven substantially with deleting any one study at a time, indicating the stability of the pooled effects. Simultaneously, there were several inevitable limitations to be put forward. First, although the multi-variable-adjusted RRs were extracted for data synthesis, the inclusive studies were observational studies that were inevitably susceptible to inherent or unmeasured confounding factors. Second, the accuracy of flavonoid subclass intake assessment was the foremost limitation of the component-based epidemiological approach. Imprecision of flavonoid intake

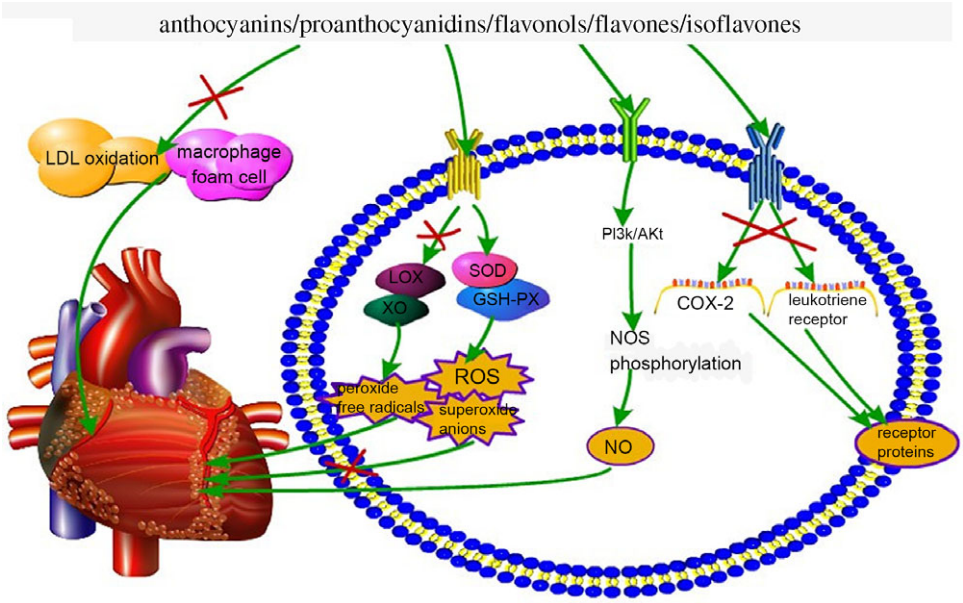

Fig. 7. The overall possible mechanisms of five flavonoid subclasses protecting against CHD. In terms of antioxidants, anthocyanins/proanthocyanidins/flavonols/flavones/isoflavones could inhibit the production and activity of peroxide free radicals, ROS and superoxide anions by decreasing the activity of LOX and XO and activating the activity of SOD and GSH-PX. In terms of anti-inflammatory effects, they could reduce LDL oxidation, macrophage foam cell formation and inhibit the genes expression of leucotriene receptor and COX-2. In addition, they could activate the PI3K/Akt pathway for NOS phosphorylation to produce NO dilation of blood vessels. Akt, protein kinase; COX-2, cyclo-oxygenase-2; GSH-PX: glutathione peroxidase; LOX, lipoxygenase; NOS, nitric oxide synthase; PI3k, phosphoinositide 3 kinase; ROS, nitric oxide synthase; SOD, superoxide dismutase; XO, xanthine oxidase.

calculation and evaluation might influence the final conclusions. Finally, flavonoids were existed in a variety of plant-derived foods, so dietary intakes of flavonoids might be under-estimated rather than over-estimated by validating FFQ.

In conclusion, this meta-analysis provided ample evidence that dietary intakes of anthocyanins, proanthocyanidins, flavonols, flavones and isoflavones were negatively correlated with CHD risk. These results were consistent with current recommendations to increase consumption of fruits and vegetables abundant in flavonoids, such as berries, grapes, soyabean and teas, would confer a lower risk of CHD. Considering that the primary prospective studies have been conducted in western countries, further studies are warranted to confirm these associations in other regions and ethnic origins.

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Z. F. and X. G. conceived and designed this study. Z. F., C. W., T. Y. and X. L. searched and checked the data. Z. F. and C. W. extracted and analysed the data. Z. F. composed the manuscript. Z. F., X. G. and L. D. have reviewed and revised the paper. All authors read and approved the final manuscript. X. G. and D. L. carefully reviewed and ratified the manuscript.

There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114521003391

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