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Title: Dietary intake of anthocyanins and risk of cardiovascular disease: a systematic review and meta-analysis of prospective cohort studies

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Abbreviations: BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio; food frequency questionnaire, FFQ; MI, myocardial infarction; MOOSE, meta-analysis of observational studies in epidemiology; PRISMA, preferred reporting items for systematic reviews and meta-analysis; RR, relative risk; SE, standard error.

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ABSTRACT

Accumulating evidence suggests flavonoid intake is associated with reduced risk of non-communicable diseases. We aimed to systematically determine and quantify the potential association between dietary anthocyanin intake and risk of cardiovascular diseases (CVD). A systematic literature search of studies reporting anthocyanin intake and risk of fatal or nonfatal CVD was performed using SCOPUS, MEDLINE, CINAHL and Cochrane Library. The relative risk (RR) or hazard ratio (HR) of highest category of anthocyanin foods were pooled in a random-effects meta-analysis. Subgroup analysis were conducted to determine possible sources of heterogeneity. The meta-analysis suggested intake of dietary anthocyanins and reduced risk of CHD (RR = 0.91, 95% CI: 0.83, 0.99; $I^2 = 12.0$, $P_h = 0.337$) and CVD mortality (RR = 0.92, 95% CI: 0.87, 0.97; $I^2 = 0.0$, $P_h = 0.584$). However, there was no relationship between the intake of these compounds and reduced risk of MI, stroke or total CVD. Subgroup analysis determined reduced risk of CHD and CVD mortality was more prominent for anthocyanidin intake, as opposed to anthocyanin or berries. Our systematic review and meta-analysis provides evidence that anthocyanins, specifically anthocyanidins, reduce the risk of CHD and CVD mortality. Further randomised controlled trials on anthocyanin intake and CVD risk factors are needed to support these findings.

INTRODUCTION

Historically, berry and cherry plant products have been widely utilized as medicinal sources to treat and prevent a number of ailments, including, but not limited to; stomach-aches, snakebites, inflammation, fevers and pains (Hummer, 2010; Stewart, 2003). More recently, there is robust epidemiological evidence that a diet rich in berry fruits is associated with a reduced risk of non-communicable and neurodegenerative diseases (Devore et al., 2012; Guo et al., 2016; Huang et al., 2016). Such health benefits are thought to be attributable to their naturally occurring flavonoids, which are further sub-classified into flavonols, flavones, flavanones, flavan-3-ols, isoflavones, and anthocyanins (Wang et al., 2014). Liu and colleagues (2017), recently demonstrated through a dose-response meta-analysis that increased intake of dietary flavonoids was associated with reduced risk of all-cause mortality. Similarly, Wang et al. (2014), reported flavonoid intake was associated with reduced risk of cardiovascular disease (CVD). However, individual flavonoids might not be equally associated with cardioprotection, given the extensive differences that exist between their bioavailability and bioactivity. Notably, berry fruits are rich in the subclass of flavonoids anthocyanins, which evidence suggests the bioavailability to be greater than previously estimated (Czank et al., 2013). Anthocyanins (from the Greek *anthos*, a flower, and *kyanos*, dark blue) are important secondary plant metabolites that occur primarily as glycosides of their aglycone anthocyanidins (Figure 1). They are water-soluble pigments often responsible for the orange, red, and blue colours in fruits, vegetables, flowers, and other tissues in plants (Delgado-Vargas et al., 2000). Hence are most abundant in commonly consumed fruits that are dark red and purple, such as blackberries, raspberries, strawberries, blueberries, red grapes and cherries (Clifford, 2000; Horbowicz et al., 2008). Accumulating evidence substantiates that anthocyanins have a putative role in overall cardiovascular health (Reis et al., 2016).

Recent *in vitro* studies have established that anthocyanin metabolites are just as bioactive, if not more, than their parent compounds (Amin et al., 2015; Keane et al., 2016). They have been demonstrated to elicit vasoprotective properties such as antioxidant, anti-inflammatory, anti-atherogenic and vasodilatory actions (Castaneda-Ovando et al., 2009; Edwards et al., 2015; Wang et al., 1999) and there is now a growing body of evidence also supports that intake of dietary anthocyanins can improve functional vascular health *in vivo* (Fairlie-Jones et al., 2017; Jennings et al., 2012). The potential underlying mechanisms for the aforementioned might involve the augmentation of endothelial-derived nitric oxide (NO) bioavailability. Anthocyanins can directly increase NO through upregulation of the endothelial nitric oxide synthase and L-arginine pathways, but also indirectly by potentiating the nitrate-nitrite-NO pathway, and minimising degradation of NO via their antioxidant actions (Edwards et al., 2015; Rocha et al., 2014). Nitric oxide is an integral molecule in regulating endothelial homeostasis and anthocyanin-rich foods have previously been demonstrated to improve endothelial function (Rodriguez-Mateos et al., 2013), which has been replicated in studies that supplemented with purified anthocyanins (Zhu et al., 2011). Accordingly, in a recent attempt to evaluate the effect of flavonoid intake on mortality, subgroup analysis determined significant decreases in risk of CVD mortality associated with the highest intake of anthocyanidins (Summary relative risk = 0.89, 95% CI: 0.83, 0.95) amongst 5 cohorts (Grosso et al., 2017). However, as highlighted by these and other authors (Grosso et al., 2017; Hooper et al., 2008), there is a need for a better understanding of the role of specific flavonoid subclasses in relation to health and disease. Moreover, there is inherent structural complexity among different flavonoids and diversity between flavonoid-containing foods and only CVD mortality was investigated (Grosso et al., 2017). Thus, conceptually it is possible that encompassing all flavonoids resulted in missing studies that evaluated anthocyanin-rich foods and the relationship between the intake of these compounds and risk of CVD incidence has not recently been determined since Wang and

colleagues (2014) previously reported significant reductions in CVD risk associated with intake of anthocyanidins (RR = 0.89, 95% CI: 0.83, 0.96; I^2 = 0.0, P_h =0.741) in a subgroup analysis of 3 studies. Therefore, the aim of the current meta-analysis was to evaluate the quantitative relationship between dietary intake of parent anthocyanidin compounds and/or anthocyanin-rich foods and the risk of CVD and related mortality from prospective cohort studies.

METHODS

Search strategy

This review followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (Stroup et al., 2000). The protocol of this systematic review was pre-registered with PROSPERO, the International Prospective Register of Systematic Reviews [registration number: CRD42018089121]. A systematic literature search was performed using SCOPUS, MEDLINE (ProQuest), CINAHL (EBSCO) and Cochrane Library from inception until January 2018. The search strategy was conducted using Medical Subject Heading (MeSH), Boolean operations and formed from existing reviews of anthocyanin-rich foods (Fairlie-Jones et al., 2017), collated in three key concepts; (i) anthocyanins, (Hashimoto et al.) cardiovascular disease and (iii) prospective study design. Furthermore, the reference list of retrieved systematic reviews and included studies were hand searched to find potential articles that could be included in the current meta-analysis.

Study selection

Studies were included for analysis if they met the following inclusion criteria: 1) were a prospective study in adults (≥ 18 years) including; prospective cohort, nested case-control and

case-cohort studies; 2) included ≥ 2 doses of anthocyanins or anthocyanin-rich foods as exposure; 3) reported fatal or non-fatal CVD events as outcome of interest [i.e. coronary heart disease, ischemic heart disease, coronary artery disease, angina, myocardial infarction, heart failure, cerebrovascular disease (ischemic stroke and haemorrhagic stroke), peripheral artery disease, CVD mortality]; 4) reported relative risk (RR) or hazard ratio (HR) and their corresponding 95% confidence intervals (or sufficient data to compute them). Titles and abstracts were independently reviewed using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia) by two researchers (RK and GH). Only full texts that were published in English or had an existing translation were retrieved and examined.

Data extraction and quality assessment

Data was independently extracted into piloted forms by two investigators (RK and KMK) in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (Stroup et al., 2000). Any discrepancies were resolved by reviewing the original article. The following data was extracted from each study: the first author's last name(s), publication date, location, the cohort name, participant's age (at baseline) and sex, the follow up period, the method of dietary assessment and anthocyanin exposure range, type of outcome, number of cases, HR or RR and their corresponding 95% CI and any covariate adjustments. Quality of each study was evaluated using the nine-star Newcastle-Ottawa scale (Stang, 2010), which assesses three major domains; selection of the study group (0–4 stars), comparability (0–2 stars) and outcome in the cohorts (0–3 stars). A maximum of 9-stars could be awarded, where 0-3, 4-6 and 7-9 stars were regarded as low, moderate and high quality, respectively.

Statistical Analysis

A random-effects meta-analysis was conducted because of unexplained heterogeneity between studies, where the common measure was RR. All included studies used COX's models, therefore HR were directly considered as RRs. The multivariate-adjusted RR and standard errors (SEs; derived from their corresponding 95% CIs) were logarithm transformed. If individual studies reported RR for multiple outcomes of CVD, or were stratified by gender, the risk estimates were pooled by fixed-effects model and the summary estimate was used in the main meta-analysis (Aune et al., 2017).

Sensitivity analysis was performed by omitting one study at a time to evaluate the potential bias and robustness of the overall risk estimate. Heterogeneity between studies was determined by χ^2 by Cochran's Q test (significance level at $P_h < 0.10$) (DerSimonian and Laird, 1986) and the I^2 statistic. For the I^2 statistic, I^2 values ≤25%, ≤50%, ≤75% and >75% indicated no, little, moderate and significant heterogeneity, respectively. Subgroup analysis was conducted to determine possible sources of heterogeneity. Subsequently, the effect of exposure, type of outcome, sex, location, sample size and follow-up length of the included studies evaluated. Potential publication bias was evaluated by Egger's test ($P < 0.10$) and visual inspection of funnel plots (Begg and Mazumdar, 1994). All statistical analysis was conducted using STATA v.15.0 (StataCorp, College Station, Texas, USA), $P < 0.05$ was considered significant unless otherwise stated.

RESULTS

Literature search

The literature search and study selection results are presented in Figure 2. There were 1689 studies initially identified by the search strategy. After exclusion of duplicates 1277 titles and

abstracts were reviewed. Seventy-six full texts were retrieved and after irrelevant articles were excluded for; wrong exposure (29), wrong study design (22),wrong outcomes (5), not complete (1), not English (2); 17 articles remained. Two additional study was identified by hand searching reference lists.

Study characteristics

The characteristics of the included studies are presented in Table 1. Nineteen studies involving 602,054 participants with more than 22,673 incidence of non-fatal or fatal CVD were included in the current meta-analysis (Cassidy et al., 2016; Cassidy et al., 2013; Cassidy et al., 2012; Goetz et al., 2016; Hirvonen et al., 2000; Hjartåker et al., 2015; Ivey et al., 2013; Jacques et al., 2015; Knekt et al., 1996; Lai et al., 2015; Larsson et al., 2013; McCullough et al., 2012; Mink et al., 2007; Mizrahi et al., 2009; Mursu et al., 2008; Ponzo et al., 2015; Sesso et al., 2007; Tresserra-Rimbau et al., 2014; Zamora-Ros et al., 2013). All studies were published between 1996-2016, with seven studies conducted in USA (Cassidy et al., 2016; Cassidy et al., 2013; Cassidy et al., 2012; Goetz et al., 2016; McCullough et al., 2012; Mink et al., 2007; Sesso et al., 2007) eleven in Europe (Hirvonen et al., 2000; Hjartåker et al., 2015; Jacques et al., 2015; Knekt et al., 1996; Lai et al., 2015; Larsson et al., 2013; Mizrahi et al., 2009; Mursu et al., 2008; Ponzo et al., 2015; Tresserra-Rimbau et al., 2014; Zamora-Ros et al., 2013) and one in Australia (Ivey et al., 2013). Five studies reported anthocyanin intake (1.9-74.6 mg/day) (Cassidy et al., 2016; Cassidy et al., 2013; Cassidy et al., 2012; Jacques et al., 2015; Tresserra-Rimbau et al., 2014), seven studies reported anthocyanidin intake (0-41 mg/day) (Goetz et al., 2016; Ivey et al., 2013; Jacques et al., 2015; McCullough et al., 2012; Mink et al., 2007; Mursu et al., 2008; Ponzo et al., 2015; Zamora-Ros et al., 2013) and seven studies reported berry intake [0-365 g/day (Hirvonen et al., 2000; Knekt et al., 1996; Lai et al., 2015; Mizrahi et al., 2009); 0-0.5 median servings/day (Hjartåker et al., 2015; Larsson et al., 2013; Sesso et al.,

2007)]. The follow-up length of the included studies ranged from 4.3 to 41 years. All studies provided covariate-adjusted risk models (e.g. age, sex, BMI, smoking). Quality scores were ≥ 6 stars (Table 1; supplemental), two studies were moderate quality (6 stars) (Hirvonen et al., 2000; Ivey et al., 2013) and all other studies considered high quality (≥ 7 stars).

Intake of anthocyanins and cardiovascular disease risk

The random-effects pooled results for fully adjusted risk estimates of subgroups of CVD (Figure 3) showed an inverse association for intake of dietary anthocyanins and reduced risk of CHD (RR = 0.91, 95% CI: 0.83, 0.99; $I^2 = 12.0$, $P_h = 0.337$) and CVD mortality (RR = 0.92, 95% CI: 0.87, 0.97) with no between-study heterogeneity ($I^2 = 0.0$, $P_h = 0.584$). However, there was no relationship between the intake of these compounds and reduced risk of MI, stroke or total CVD. Subgroup analyses can be found in (Table 2; Supplemental). Reduced risk of CHD (RR = 0.84, 95% CI: 0.71, 0.98; $I^2 = 31.6$, $P_h = 0.227$) and CVD mortality (RR = 0.92, 95% CI: 0.87, 0.97; $I^2 = 0.0$, $P_h = 0.491$) remained significant for anthocyanidin studies only, as opposed to studies which determined intake of berries or anthocyanins. Subgroup analysis also determined risk estimates to be significant for studies conducted in the USA for both CHD (RR = 0.84, 95% CI: 0.71, 0.98; $I^2 = 31.6$, $P_h = 0.227$) and CVD mortality (RR = 0.89, 95% CI: 0.83, 0.96; $I^2 = 0.0$ $P_h = 0.691$). Risk of MI, stroke and total CVD remained non-significant when stratified by exposure, sex, location, follow up and sample size. Stroke was further stratified by type (Figure 4), but no association was found between the intake of these compounds and reduced risk of cerebral infarction, ischaemic or hemmorrhagic strokes.

Publication bias and sensitivity analysis

Egger's test suggested the absence of significant publication for all studies based on each type of CVD ($P \geq 0.127$). This was confirmed by visual inspection of the funnel plots (Figure 1; supplemental) which showed no substantial asymmetry. Overall, sensitivity analysis determined no materially different risk estimates indicating stable results between risk estimates of studies included in the pooled estimates (Figure 1; supplemental).

DISCUSSION

The current meta-analysis of nineteen prospective cohort studies is the largest, most comprehensive and contemporary to evaluate the association between dietary anthocyanin intake and risk of CVD. Our findings indicate that dietary intake of anthocyanins is inversely associated with CHD and CVD mortality (Figure 3), which is consistent with the findings of others (Grosso et al., 2017; Wang et al., 2014). Conversely, we found no relationship between the intake of these compounds and the risk of Stroke, MI or total CVD. This is surprising given that there is commonality in the aetiology of CVDs and a recent meta-analysis of randomised controlled trial showed and berry supplementation improved risk factors of CVD; specifically lipid profiles, systolic and diastolic blood pressure, which the authors speculate might be, at least partly, attributable to the anthocyanin content (Luís et al., 2018). Moreover, there is a growing body of evidence that increased flavonoid intake is associated with reduced risk of stroke (Tang et al., 2016) and total CVD (Wang et al., 2014). However, most of the prior epidemiologic evidence on flavonoids and CVD risk, specifically stroke, has focused on flavanones, flavones, flavonols, and flavan-3-ols (Tang et al., 2016) that might have different bioactivities compared to anthocyanins. Conversely, there are a number of weaknesses in the available data. Firstly, the majority of included studies in the current meta-analysis used FFQs to assess dietary intake of berries which have previously been reported to be less reliable to

assess berry intake compared to other foods (Bjelke, 1974). Secondly, most included studies used single assessments to categorise dietary anthocyanin intake, but this could likely change during the study (Jacques et al., 2015). Thirdly, although determination of anthocyanin/anthocyanidin intake is likely to be more comprehensive than intake of berries alone, there is the possibility of measurement error and misclassification. Furthermore, there is inherent difficulty in determining the intake of these compounds because the databases have missing foods and limited information on retention of these compounds following cooking (Zamora-Ros et al., 2011), and some studies would likely have used outdated databases. Thus, the different associations between CVDs might be confounded by suboptimal quantification of dietary intake of berries and/or anthocyanins, but could also be because of the limited number of studies found examining MI and total CVD.

In the current meta-analysis, we found higher anthocyanin intake was associated with a 9% and 8% reduced risk of CHD and CVD mortality, respectively. This is of great interest because CVD remains the most common cause of death worldwide, accounting for 45% of all deaths in Europe (Townsend et al., 2016) and is estimated to cost healthcare services £15.7 billion in the UK alone (Luengo-Fernandez et al., 2006). Our findings are of a similar magnitude to a recent meta-analysis that found that berry intake was associated with a significant reduction in all-cause mortality (RR = 0.92, 95% CI: 0.88, 0.97; I^2 = 8.0, P_h =0.34) (Aune et al., 2017). Moreover, Guo and colleagues (2016) previously reported that risk of type II diabetes was reduced (5%) by increasing anthocyanin intake (7.5 mg/day; RR = 0.95; 95% CI: 0.93, 0.98) and berry intake (17 g/day; RR = 0.95; 95% CI: 0.91, 0.99), suggesting these compounds have a number of health benefits.

Conversely, there was no relationship between intake of anthocyanins and risk of stroke. Further stratification by types of stroke because of possible differences in underlying mechanisms (Figure 4) revealed there was no relationship between the intake of these

compounds and cerebral infarction, ischaemic or hemorrhagic strokes. The reasons for the different risk estimates between anthocyanin intake and CHD versus stroke remains unknown, but might be because 1) anthocyanins do not circulate the brain in large enough doses to elicit neurovascular protection, or 2) the cerebrovasculature is more susceptible to atherosclerosis because of the smaller vessel diameters (Mursu et al., 2008). Our findings are consistent with a recent meta-analysis that reported highest berry intake was not associated with reduced risk of stroke (RR = 0.98, 95% CI: 0.86, 1.12) (Aune et al., 2017). The majority of included stroke studies in the current meta-analysis had berries as the exposure (Table 2; supplemental). Berries are a major source of anthocyanins, but these compounds are not uniformly distributed between these and other sources (Clifford, 2000; Horbowicz et al., 2008). In a subgroup-analysis we found more prominent relationships between anthocyanidins and reduced risk of CHD (16%) and CVD mortality (8%). Interestingly, because the included studies reported sources other than berries, such as red wine and cherries to significantly contribute to overall anthocyanin intake (Ponzo et al., 2015; Tresserra-Rimbau et al., 2014), perhaps the specific anthocyanins needs more careful consideration.

A strength of this meta-analysis was the inclusion of multivariate-adjusted risk models that adjusted for important confounding variables. However, a limitation of the included studies is that intake of anthocyanins is positively correlated to fruit and vegetable intake and the beneficial nutrients associated with these, such as other flavonoids, vitamins, carotenoids and fibre (Zamora-Ros et al., 2013). Thus, because of multicollinearity, and because only some studies adjusted for some of these as covariates (Table 1), it is not possible to isolate whether the observed reduction in risk estimates is solely in response to intake of dietary anthocyanidins and not other bioactive compounds. We also found varying risk estimates for study locations; for example, reduced risk of CHD and CVD mortality only remained significant in the USA (Table 2; supplemental). It is plausible that this is attributable to differing dietary patterns and

inclusion of a diet richer in anthocyanidins (Wu et al., 2006; Zamora-Ros et al., 2011) and altered the bioavailabity of these compounds *in vivo,* especially in relation to interactions between diet and host microbiome (D'Archivio et al., 2010). It should also be acknowledged that other bioactive compounds, not just anthocyanidins, are likely to have synergistic interactions (Keane et al., 2016; Kirakosyan et al., 2010) and might not be as beneficial in isolation. Nonetheless, recent *in vitro* and human studies of anthocyanins and their relevant metabolites suggests that these compounds are responsible for some degree of cardioprotection (Fairlie-Jones et al., 2017; Reis et al., 2016). Although teleologically intuitive, this concept and the results of this meta-analysis should be interpreted cautiously given that previous studies have failed to demonstrate reduced risk of chronic diseases with antioxidant supplementation (Bjelakovic et al., 2012). Therefore, future randomised controlled trials should determine the longitudinal effects of anthocyanin supplementation on CHD and CVD risk factors to support the findings of the current meta-analysis.

In summary, this systematic review and meta-analysis provides further support that anthocyanins, specifically anthocyanidins, reduce the risk of CHD and CVD mortality, which from a public health perspective might help reduce the associated socioeconomic burdens. However, there was no relationship between the intake of these compounds and reduced risk of MI, stroke or total CVD; moreover, whether particular groups of anthocyanin-containing foods, such as berries, seem to be more beneficial than other fruits or vegetables remains to be established. Until more precise conclusions can be made, we recommend that dietary intake should include anthocyanidin-rich sources adjunct a varied diet of fruit and vegetables rich in other bioactive compounds. Further longitudinal randomised controlled trials determining the effect of anthocyanin intake on cardiovascular risk factors are needed to support the findings of the current meta-analysis.

Author Contributions

The authors' responsibilities were as follows—RK, KMK, JKL and GH: conceived and designed the systematic review and meta-analysis; RK, KMK and GH: acquired and interpreted the results; RK and GH: drafted the manuscript; and all authors: critically revised the meta-analysis and approved submission of the final manuscript. None of the authors reported a conflict of interest related to the study.

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Table 1. Characteristics of nineteen prospective cohort studies on dietary anthocyanin intake and risk of cardiovascular disease

Reference	Country	Study cohort and no. of participants	Sex	Age range or mean (years)	Follow-up duration (years)	Exposure and assessment method	Outcome and no. of cases	Covariate adjustments	Study quality
Cassidy et al. (2012)	USA	Nurses' Health Study 69622	Females	30-55	14	Anthocyanins FFQ	Ischemic stroke 943 Hemorrhagic stroke 253 Stroke 1803	Age, BMI, physical activity, alcohol consumption, energy intake, use of multivitamin supplements, use of aspirin, menopausal status, smoking and history of Type 2 diabetes, coronary heart disease, hypercholesterolemia, or hypertension.	7
Cassidy et al. (2013)	USA	Nurses' Health Study II 93600	Females	25-42	18	Anthocyanins FFQ	MI/CHD 405	Age, BMI, physical activity, alcohol consumption, energy intake, cereal fibre intake, fat intake, caffeine intake, use of aspirin, menopausal status, postmenopausal hormone use, oral contraceptive use, smoking, and family history of MI.	7
Cassidy et al. (2016)	USA	Health Professionals Follow-Up Study 43880	Males	32-81	24	Anthocyanins FFQ	Total MI 863 Nonfatal MI 482 Fatal MI 379 Stroke 346 Ischemic stroke 200	BMI, physical activity, alcohol consumption, smoking, marital status, history of hypertension, history of hypercholesterolemia, quintiles of energy intake, cereal fibre, fat intake and folate, and family history of MI.	7

							Hemorrhagic stroke 48		
Goetz et al. (2016)	USA	Reasons for Geographic and Racial Differences in Stroke 16678	Males/Females	>45	6.06	Anthocyanidins FFQ	MI/CHD 589	Age, energy intake, sex, physical activity, smoking demographic factors (race and region of residence), socioeconomic factors (household income and educational attainment), energy intake from sweetened foods and beverages, reported beer, liquor and fat intake.	7
Hirvonen et al., (2000)	Finland	Alpha-Tocopherol, Beta-Carotene Cancer Prevention 26593	Males	50-69	5-8	Berries FFQ	Cerebral infarction 736 Subarachnoid haemorrhage 83 Intracerebral haemorrhage 95	Age, BMI, height, supplementation group, systolic and diastolic blood pressures, serum total cholesterol, serum HDL cholesterol, smoking-years, number of cigarettes daily, history of diabetes or coronary heart disease, alcohol intake, and education.	6
Hjartaker et al., (2015)	Norway	Norwegian Migrant cohort 10718	Males	33-73	41	Berries FFQ	CVD mortality 4595	BMI, physical activity, beer, spirits, coffee, socioeconomic status (professional, administration, agricultural, industrial and other) and total smoking.	7
Ivey et al., (2013)	Australia	The Calcium Intake Fracture Outcome Age Related Extension Study	Females	>75	5	Anthocyanidin FFQ	CVD mortality 64	Age, energy intake, BMI, previous atherosclerotic vascular disease, energy expended in physical activity, previous diabetes, anti-hypertensive medication use, history of smoking and intakes of saturated	6

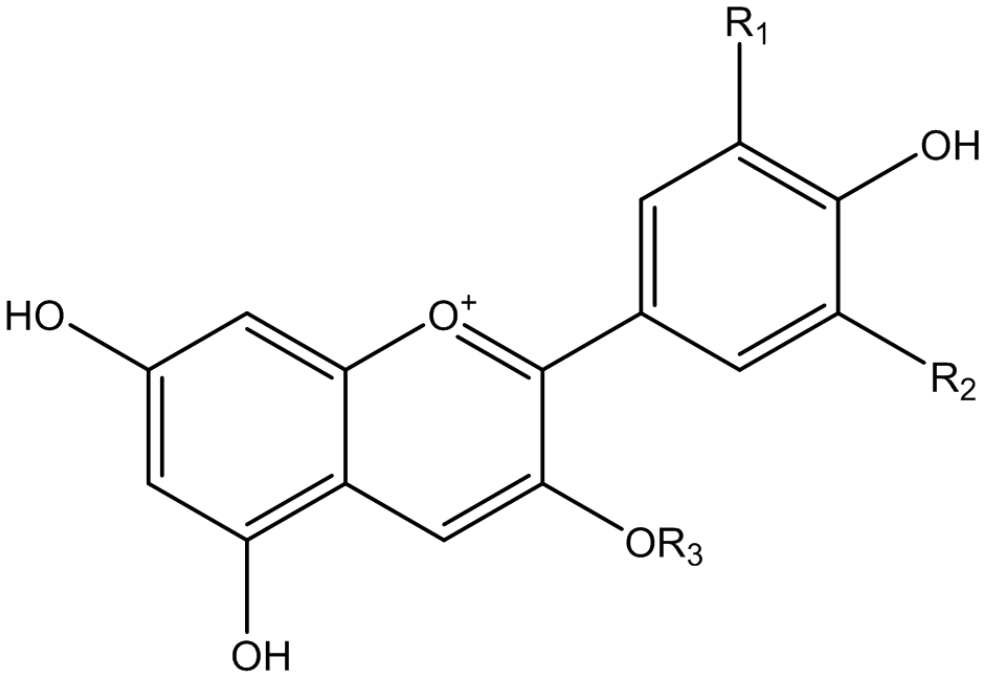
		1063						fat, fibre, protein, starch, vitamin C and alcohol at baseline. Sensitivity analysis was performed by repeating logistic regression analysis in participants without previous atherosclerotic vascular disease and diabetes at baseline.	
Jacques et al., (2015)	USA	Framingham Heart Study 2880	Males/Females	28-62	20	Anthocyanins FFQ	Total CVD 518 CHD 261	Age, sex, current smoking status, BMI, total energy intake and fruit/vegetable intake.	8
Knekt et al., (1996)	Finland	The mobile clinic of the Finnish Social Insurance Institution 2748	Males	35-69	26	Berries Dietary history interview	CHD 324	Age, BMI, smoking, serum cholesterol and hypertension.	7
Knekt et al., (1996)	Finland	The mobile clinic of the Finnish Social Insurance Institution 2385	Females	35-69	26	Berries Dietary history interview	CHD 149	Age, BMI, smoking, serum cholesterol and hypertension.	7
Lai et al., (2015)	UK	UK Women's Cohort Study 30458	Females	30-69	16.7	Berries FFQ	CHD 138 Stroke 148 Total CVD 286	Age, BMI, physical activity, smoking status, socio-economic status, alcohol intake, total vegetable intake, and mutual adjustments for fruits that are not in the exposure category.	7

Larsson et al. (2013)	Sweden	Swedish Mammography Cohort and the Cohort of Swedish Men 74961	Males/Females	45-83	10.2	Berries FFQ	Cerebral infarction 3159 Intracerebral haemorrhage 435 Subarachnoid hemorrhage 347 Stroke 4089	Age, sex, BMI, smoking status and pack-years of smoking, education, total physical activity, aspirin use, history of hypertension, diabetes, family history of myocardial infarction, and intakes of total energy, alcohol, coffee, fresh red meat, processed meat, fish, total vegetable consumption and mutually adjusted for total fruit consumption.	8
McCullough et al., (2012)	USA	Cancer Prevention Study II Nutrition Cohort 98469	Males/Females	69.5	7	Anthocyanidins FFQ	CVD mortality 2771	Age, smoking, beer and liquor intake, history of hypertension, history of cholesterol, family history of myocardial infarction, BMI, physical activity, energy intake, aspirin use, hormone replacement therapy (in women only), and sex (in combined model only).	8
Mink et al., (2007)	USA	Iowa Women's Health Study 34489	Females	55-69	16	Anthocyanidins FFQ	CVD mortality 2361 CHD mortality 1329 Stroke 469	Age, energy intake, marital status, education, blood pressure, diabetes, BMI, waist-to-hip ratio, physical activity, smoking, and oestrogen use.	7
Mizrahi et al., (2009)	Finland	Finnish Mobile Clinic Health Examination Survey 3932	Males/Females	40-74	24	Berries Dietary history interview	Intracerebral haemorrhage 58 Ischaemic strokes 335	Age, sex, BMI, smoking, physical activity, serum cholesterol level, blood pressure and energy intake.	8

							Stroke 625		
Mursu et al., (2008)	Finland	Kuopio Ischaemic Heart Disease Risk Factor Study 1950	Males	42-60	15.2	Anthocyanidins 4-day food diary	Ischaemic stroke 102 CVD Mortality 153	Age, examination years, BMI, systolic blood pressure, hypertension medication, serum HDL- and LDL-cholesterol, serum TAG, maximal oxygen uptake, smoking, CVD in family, diabetes, alcohol intake, energy-adjusted intake of folate and vitamin E, total fat (percentage of energy) and saturated fat intake (percentage of energy).	7
Ponzo et al., (2015)	Italy	Local Health Units of the province of Asti 1658	Males/Females	45-65	12	Anthocyanidins FFQ	CVD Mortality 84 Total CVD 125	Age, sex, BMI, education, living in a rural area, METs (hour/week), fibre, and saturated fatty acid intakes, alcohol intake, smoking, values of systolic and diastolic blood pressure, total and HDL cholesterol, fasting glucose, CRP, statin and aspirin use.	7
Sesso et al., (2007)	USA	Women's Health Study 38176	Females	54	10.1	Strawberries FFQ	MI 289 Stroke 339 CVD mortality 204 Total CVD 1004	Age, randomized aspirin treatment, randomized vitamin E treatment, randomized beta-carotene treatment, and total energy intake, body mass index, exercise, alcohol intake, smoking, post-menopausal hormone use, and parental history of myocardial infarction 60 years, plus clinical factors: hypertension, hypercholesterolemia, and diabetes, plus dietary components related to	7

								strawberry intake: fruit and vegetables, fibre, folate, vitamin C, potassium, saturated fat, and total flavonoid intake.	
Tressera-Rimbau et al., (2014)	Spain	Prevención con Dieta Mediterránea 7172	Males/Females	66	4.3	Anthocyanins FFQ	Total CVD 273	Age, sex, smoking, BMI, alcohol, energy, physical activity, family history of CVD, aspirin use, antihypertensive drugs, cardiovascular drugs, and diabetes status, plus intake of proteins, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and cholesterol.	7
Zamora-Ros et al., (2013)	Spain	European Prospective Investigation into Cancer and Nutrition 40622	Males/Females	29-70	13.6	Anthocyanidins Computerized diet history questionnaire	CVD mortality 416	Age, sex, BMI, education level, physical activity, tobacco smoking, alcohol lifetime, total energy, vitamin C and fibre intakes.	7

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; HDL, high-density lipoproteins; LDL, low-density lipoproteins; food frequency questionnaire, FFQ; METs, metabolic equivalents; MI, myocardial infarction.



Anthocyanin	R_1	R_2	R_3
Cyanidin	OH	H	H
Delphinidin	OH	OH	H
Malvidin	OCH_3	OCH_3	H
Pelargonidin	H	H	H
Peonidin	OCH_3	H	H
Petunidin	OCH_3	OH	H
Cyanidin-3-glucoside	OH	H	Glc
Delphinidin-3-glucoside	OH	OH	Glc
Malvidin-3-glucoside	OCH_3	OCH_3	Glc
Pelargonidin-3-glucoside	H	H	Glc
Peonidin-3-glucoside	OCH_3	H	Glc
Petunidin-3-glucoside	OCH_3	OH	Glc

Figure 1. Major anthocyanidin compounds and their corresponding anthocyanin glycosides. Glc = glucose.

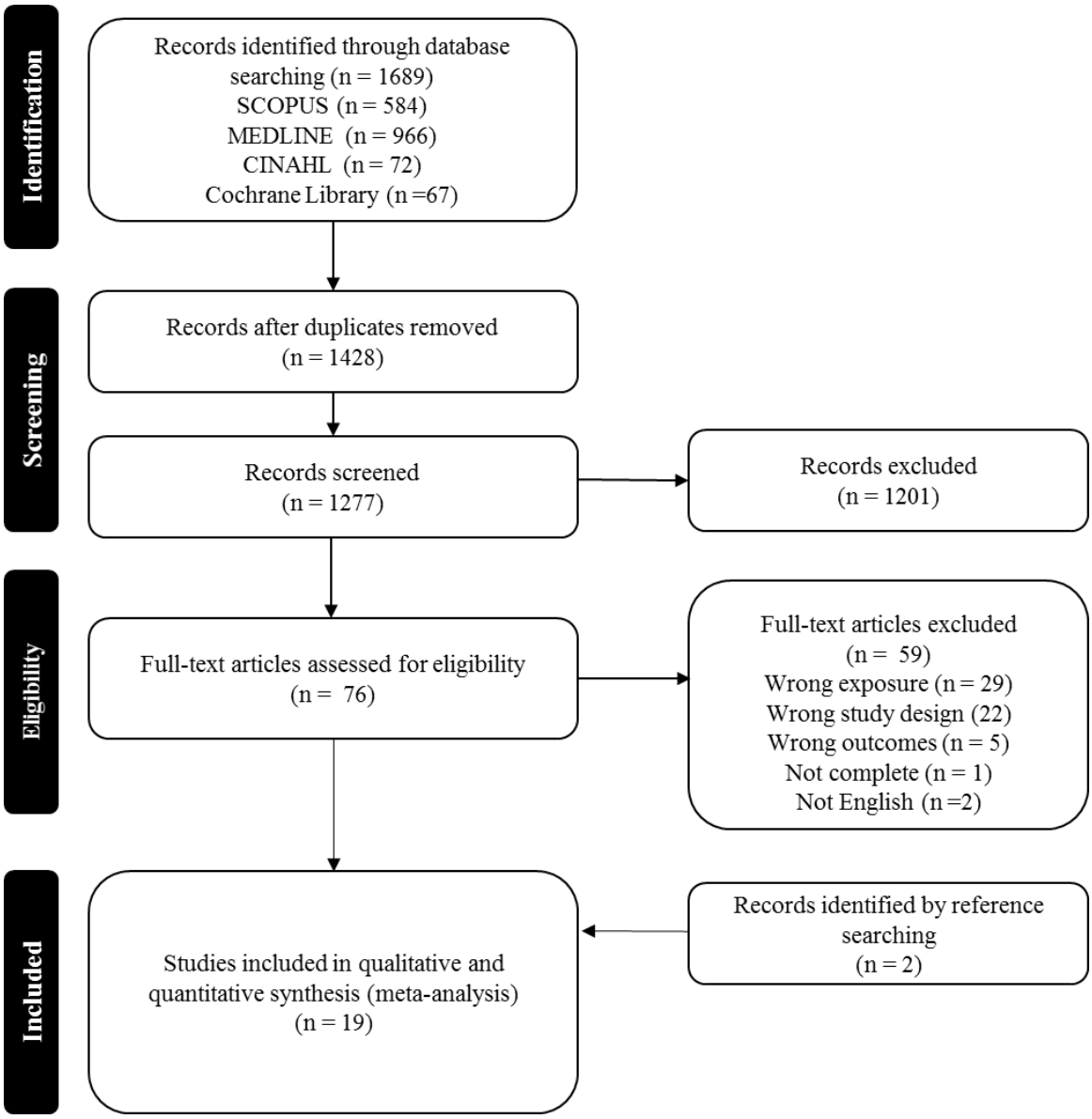


Figure 2. PRISMA flow chart for literature search and study selection.

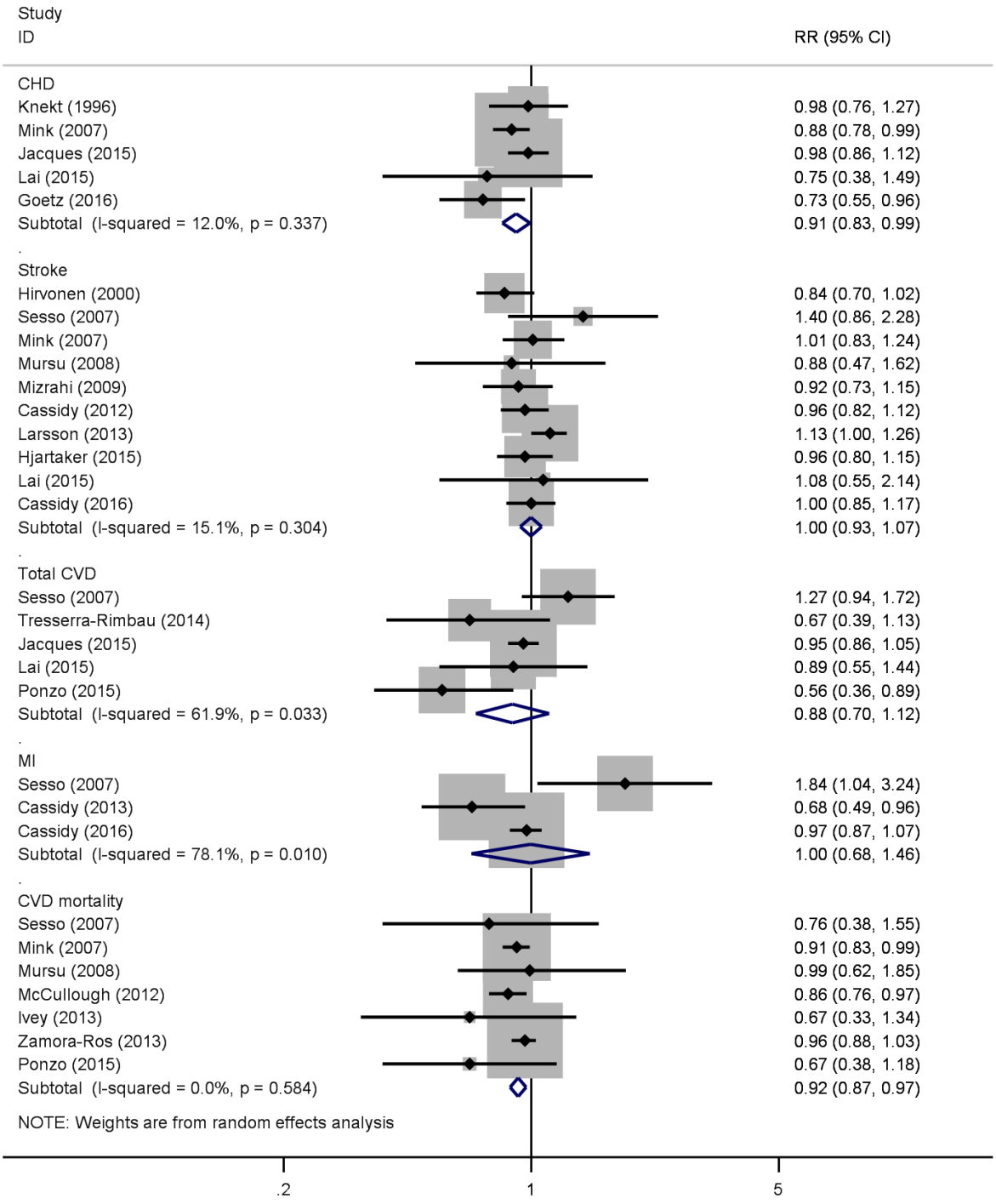


Figure 3. Random-effects analysis of fully-adjusted risk estimates of cardiovascular diseases (CVD) by highest versus lowest dietary anthocyanin intake. Grey shaded area represents proportional weighting and the diamond represent summary relative risk for coronary heart disease (CHD); stroke; total CVD; myocardial infarction (MI) and CVD mortality, respectively.

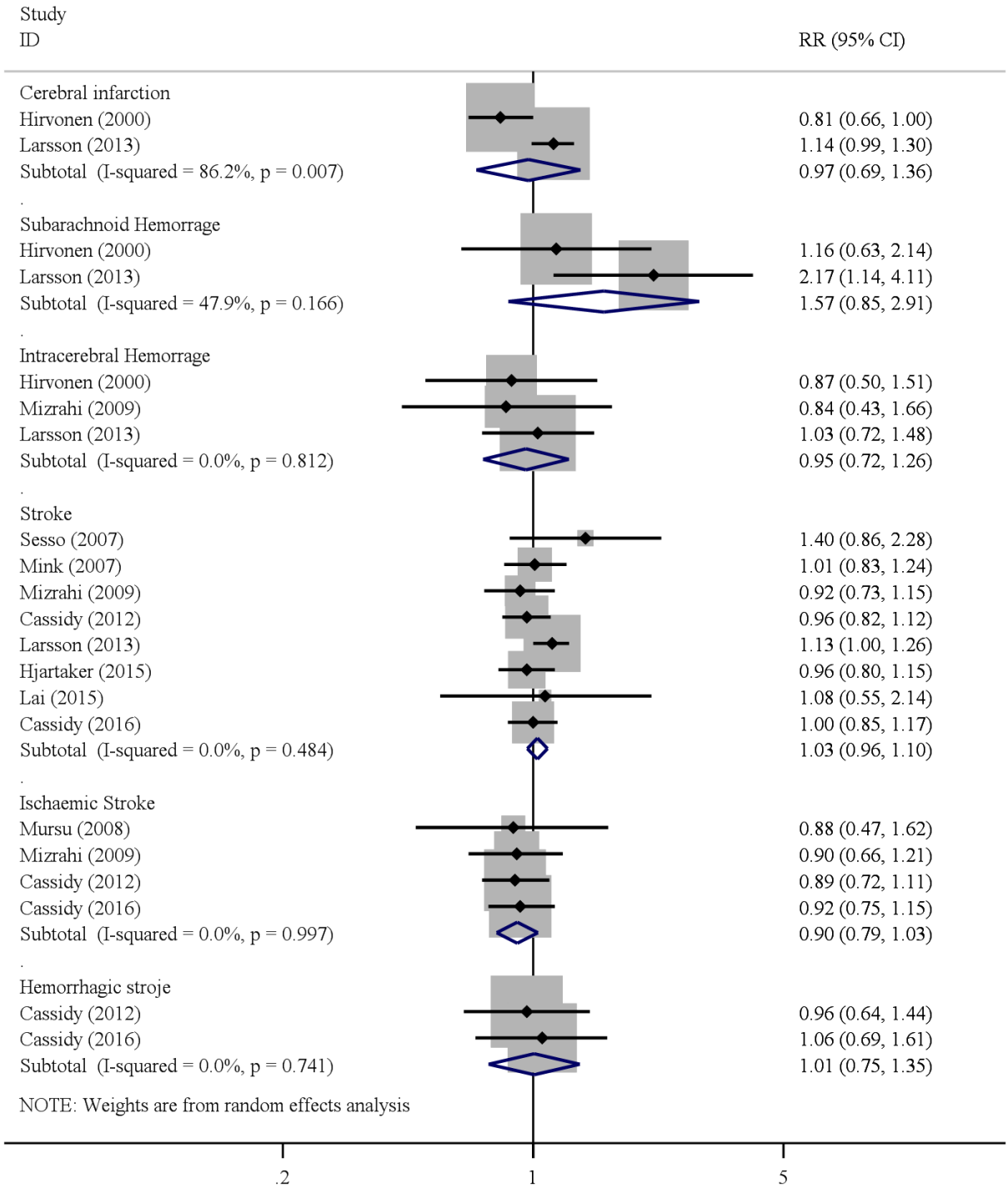


Figure 4. Random-effects analysis of fully-adjusted risk estimates of stroke by highest versus lowest dietary anthocyanin intake. Grey shaded area represents proportional weighting and the diamond represent summary relative risk for stroke subgroups.

Supplemental Table 1. Newcastle-Ottawa scale quality assessment of included studies

Study	Representativeness of exposed cohort	Selection of unexposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was present at start of study	Control for important factors or additional factors	Outcome assessment	Follow-up long enough for outcomes to occur†	Adequacy of follow-up of cohorts†	Total quality scores
Cassidy et al. (2012)	–	★	–	★	★★	★	★	★	7
Cassidy et al. (2013)	–	★	–	★	★★	★	★	★	7
Cassidy et al. (2016)	–	★	–	★	★★	★	★	★	7
Goetz et al. (2016)	★	★	–	★	★★	★	–	★	7
Hirvonen et al., (2000)	–	★	–	★	★★	★	–	★	6
Hjartaker et al., (2015)	–	★	–	★	★★	★	★	★	7
Ivey et al., (2013)	–	★	–	★	★★	★	–	★	6
Jacques et al., (2015)	★	★	–	★	★★	★	★	★	8
Knekt et al., (1996)	★	★	–	★	★	★	★	★	7
Knekt et al., (1996)	★	★	–	★	★	★	★	★	7
Lai et al., (2015)	–	★	–	★	★★	★	★	★	7
Larsson et al. (2013)	★	★	–	★	★★	★	★	★	8
McCullough et al., (2012)	★	★	–	★	★★	★	–	★	7
Mink et al., (2007)	–	★	–	★	★★	★	★	★	7
Mizrahi et al., (2009)	★	★	–	★	★★	★	★	★	8

Mursu et al., (2008)	−	☆	−	☆	☆☆	☆	☆	☆	7
Ponzo et al., (2015)	−	☆	−	☆	☆☆	☆	☆	☆	7
Sesso et al., (2007)	−	☆	−	☆	☆☆	☆	☆	☆	7
Tressera-Rimbau et al., (2014)	☆	☆	−	☆	☆☆	☆	−	☆	7
Zamora-Ros et al., (2013)	☆	☆	−	☆	☆	☆	☆	☆	7

†(follow-up of ≥ 10 years and >80% assigned one star each)

Supplemental Table 2. Subgroup analysis of different cardiovascular diseases

Subgroup	Summary RR (95% CI)	Heterogeneity		No. of cohorts	References
		P_h value	I^2 (%)		
Coronary Heart Disease					
Exposure					
Berries	0.95 (0.75, 1.21)	0.473	0.0	2	[1, 2]
Anthocyanidins	0.84 (0.71, 0.98)	0.227	31.6	2	[3, 4]
Anthocyanins	0.98 (0.86, 1.12)	−	−	1	[5]
Sex					
Males	1.21 (0.89, 1.64)	−	−	1	[1]
Females	0.81 (0.65,1.01)	0.264	24.8	3	[1-3]
Males/Females	0.87 (0.65, 1.15)	0.061	71.5	2	[4, 5]
Location					
Europe	0.97 (0.87, 1.09)	0.751	0.0	3	[1, 2, 5]
USA	0.84 (0.71, 0.98)	0.227	31.6	2	[3, 4]
Follow up					
≥ 10 years	0.93 (0.85, 1.01)	0.580	0.0	4	[1-3, 5]
< 10 years	0.73 (0.55, 0.96)	−	−	1	[4]
Sample size					
≥ 10000	0.85 (0.76,0.95)	0.449	0.0	3	[2-4]
< 10000	0.98 (0.87, 1.10)	1.000	0.0	2	[1, 5]
CVD mortality					
Exposure					
Berries	0.76 (0.38, 1.55)	−	−	1	[6]
Anthocyanidins	0.92 (0.87, 0.97)	0.491	0.0	6	[3, 7-11]
Sex					
Males	0.92 (0.79, 1.07)	0.772	0.0	2	[7, 8]
Females	0.89 (0.82, 0.96)	0.603	0.0	4	[3, 6, 8, 9]
Males/Females	0.91 (0.81, 1.01)	0.176	42.4	3	[8, 10, 11]
Location					
Australia	0.67 (0.33, 1.34)	−	−	1	[9]
Europe	0.95 (0.88, 1.03)	0.464	0.0	3	[7, 10, 11]
USA	0.89 (0.83, 0.96)	0.691	0.0	3	[3, 6, 8]
Follow up					

≥ 10 years	0.92 (0.87, 0.97)	0.562	0.0	6	[3, 6-8, 10, 11]
< 10 years	0.67 (0.33, 1.34)	–	–	1	[9]
Sample size					
≥ 10000	0.92 (0.87, 0.97)	0.451	0.0	4	[3, 6, 8, 10]
< 10000	0.78 (0.55, 1.10)	0.552	0.0	3	[7, 9, 11]
Myocardial infarction					
Exposure					
Berries	1.84 (1.04, 3.24)	–	–	1	[6]
Anthocyanins	0.84 (0.60, 1.18)	0.048	74.5	2	[12, 13]
Sex					
Males	0.97 (0.87, 1.07)	–	–	1	[13]
Females	1.09 (0.41, 2.88)	0.003	88.5	2	[6, 12]
Location					
USA	1.00 (0.68, 1.46)	0.010	78.1	3	[6, 12, 13]
Follow up					
≥ 10 years	1.00 (0.68, 1.46)	0.010	78.1	3	[6, 12, 13]
Sample size					
≥ 10000	1.00 (0.68, 1.46)	0.010	78.1	3	[6, 12, 13]
Stroke					
Exposure					
Berries	1.00 (0.88, 1.14)	0.076	49.9	6	[2, 6, 14-17]
Anthocyanidins	1.00 (0.82, 1.21)	0.678	0.0	2	[3, 7]
Anthocyanins	0.98 (0.88, 1.09)	0.720	0.0	2	[13, 18]
Sex					
Males	0.94 (0.85, 1.04)	0.564	0.0	4	[7, 13, 14, 17]
Females	1.06 (0.89, 1.27)	0.478	0.0	3	[2, 3, 6]
Males/Females	1.04 (0.86, 1.27)	0.114	60.0	2	[15, 16]
Location					
Europe	0.98 (0.87, 1.10)	0.132	41.1	6	[2, 7, 14-17]
USA	1.00 (0.91, 1.10)	0.551	0.0	4	[3, 6, 13, 18]
Follow up					
≥ 10 years	1.03 (0.96, 1.09)	0.567	0.0	9	[2, 3, 6, 7, 13, 15-18]
< 10 years	0.84 (0.70, 1.01)	–	–	1	[14]

Sample size					
≥ 10000	1.00 (0.93, 1.09)	0.201	28.5	8	[2, 3, 6, 13, 14, 16-18]
< 10000	0.92 (0.74, 1.13)	0.895	0.0	2	[7, 15]
Total CVD					
Exposure					
Berries	1.12 (0.80, 1.56)	0.220	33.5	2	[2, 6]
Anthocyanidins	0.56 (0.36, 0.88)	–	–	1	[11]
Anthocyanins	0.88 (0.67, 1.17)	0.206	37.5	2	[5, 19]
Sex					
Females	1.12 (0.80, 1.56)	0.220	33.5	2	[2, 6]
Males/Females	0.75 (0.52, 1.09)	0.041	68.7	3	[5, 11, 19]
Location					
Europe	0.80 (0.61, 1.04)	0.094	53.1	4	[2, 5, 11, 19]
USA	1.27 (0.94, 1.72)	–	–	1	[6]
Follow up					
≥ 10 years	0.92 (0.71, 1.19)	0.031	66.1	4	[2, 5, 6, 11]
< 10 years	0.67 (0.39, 1.13)	–	–	1	[19]
Sample size					
≥ 10000	1.12 (0.80, 1.56)	0.220	33.5	2	[2, 6]
< 10000	0.75 (0.52, 1.09)	0.041	68.7	3	[5, 11, 19]

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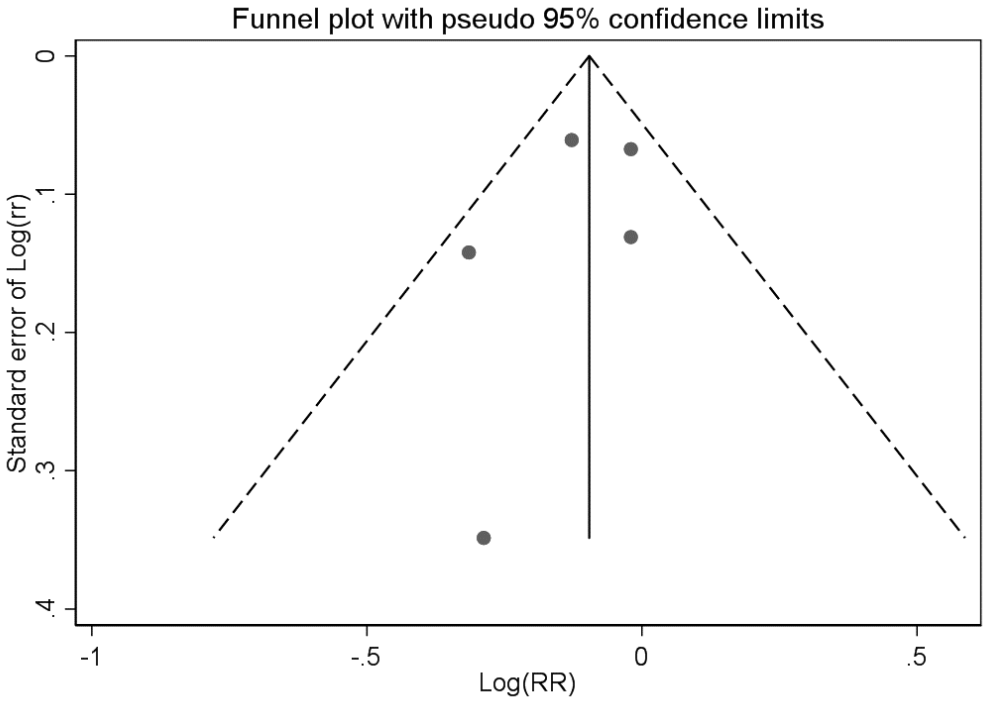
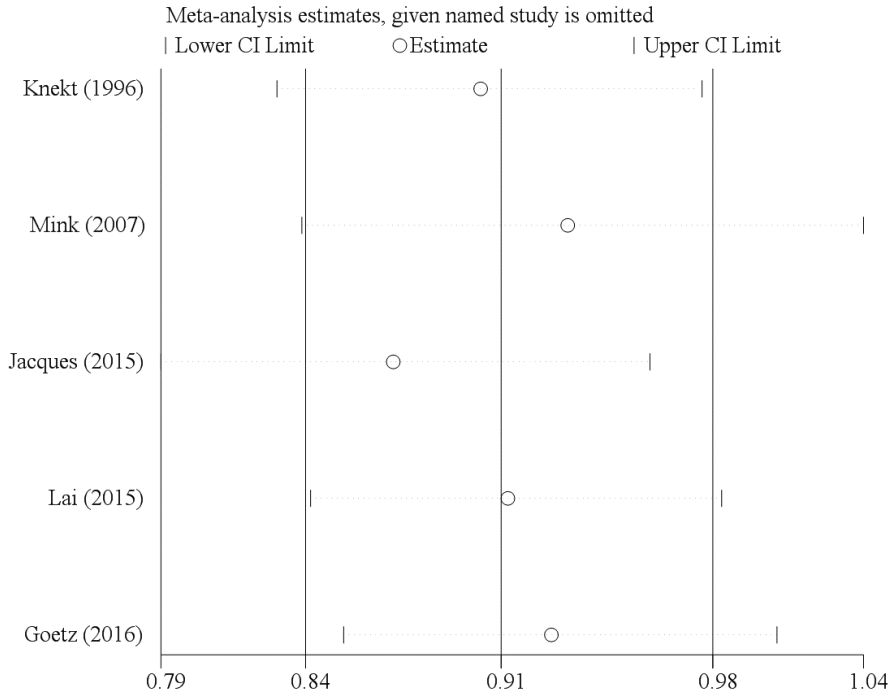
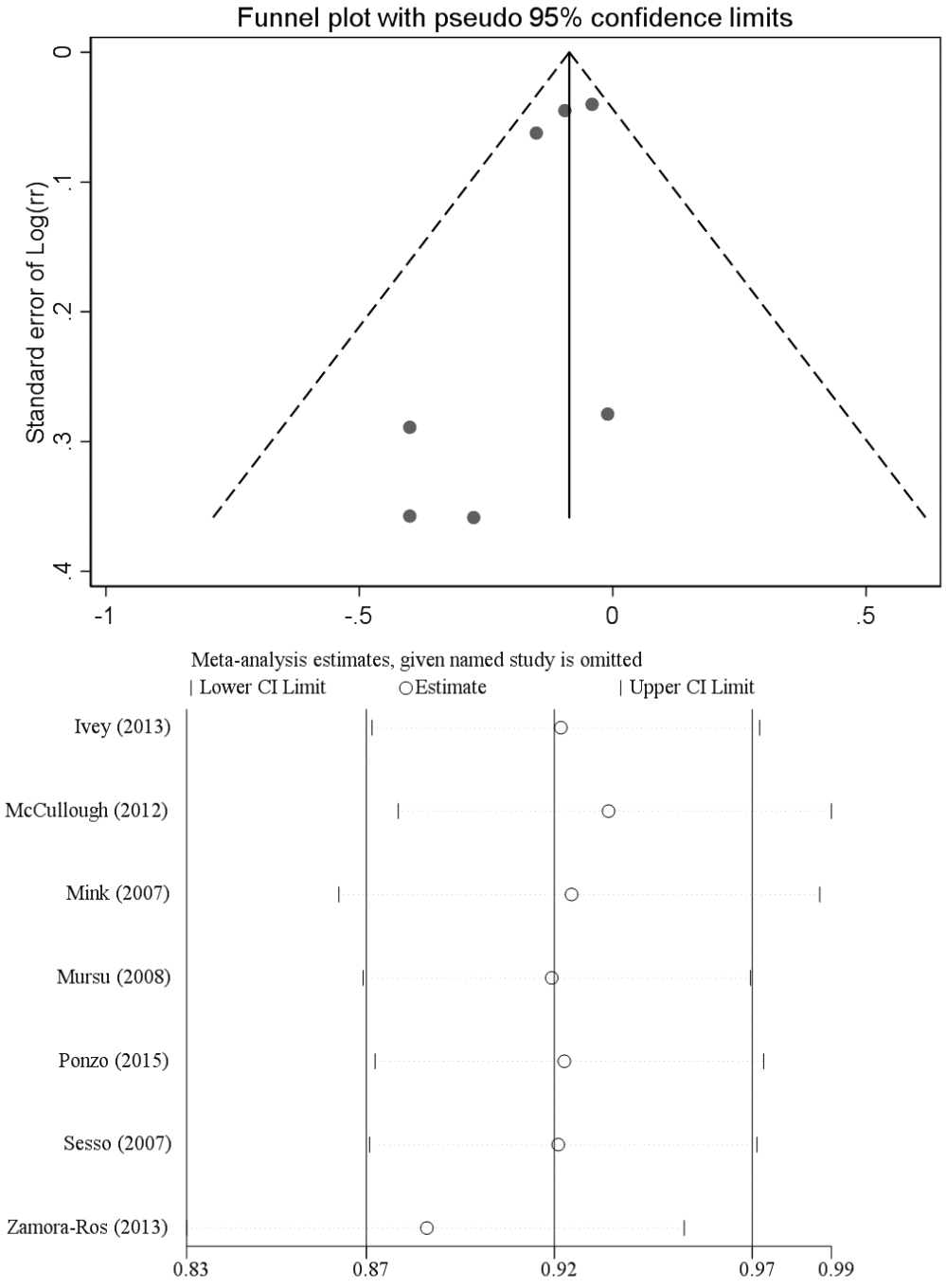




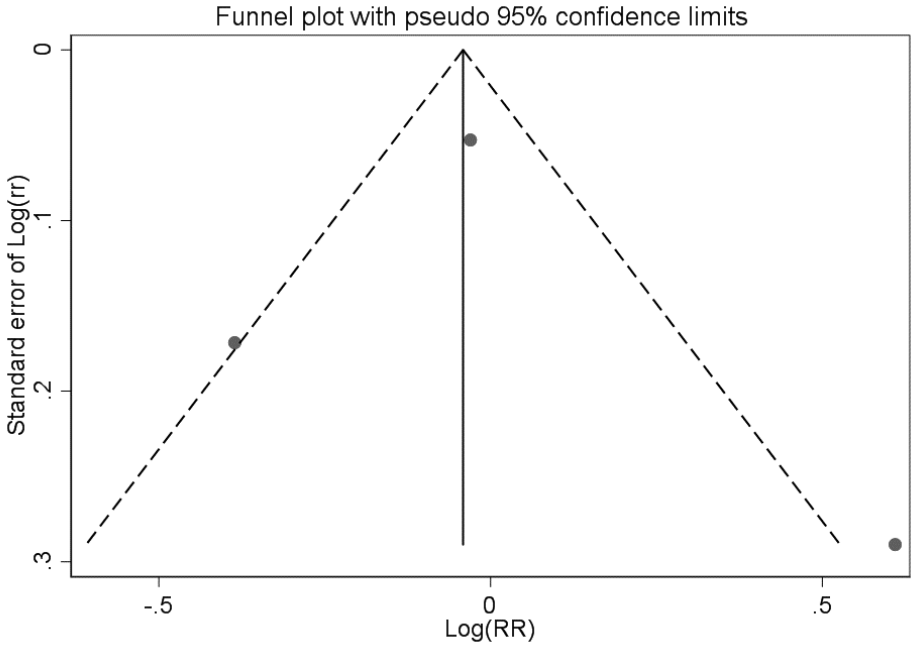
Study omitted	Estimate	[95% Conf.	Interval]
Knekt (1996)	.9013294	.82895738	.98001987
Mink (2007)	.93227661	.83777159	1.0374422
Jacques (2015)	.87026638	.78767717	.96151519
Lai (2015)	.91100657	.84086025	.9870047
Goetz (2016)	.92645848	.85263187	1.0066775
Combined	.90860584	.83910189	.98386689

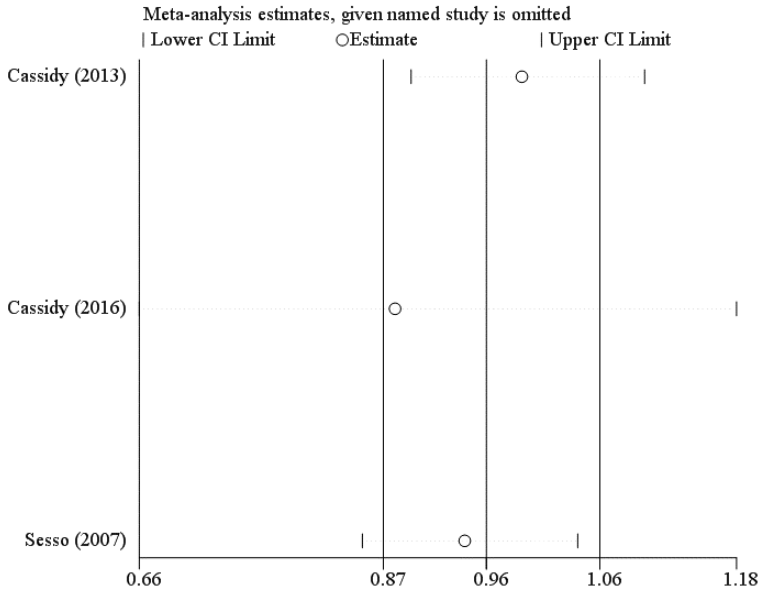
Supplemental Figure 1a. Funnel plot and sensitivity analysis for anthocyanin intake and risk of CHD.



Study omitted	Estimate	[95% Conf.	Interval]
Ivey (2013)	.9195078	.87267137	.96885794
McCullough (2012)	.93135935	.87917066	.986646
Mink (2007)	.92217618	.86443639	.9837727
Mursu (2008)	.91726065	.87045699	.96658093
Ponzo (2015)	.92036879	.87342101	.96984005
Sesso (2007)	.91885656	.87205416	.96817088
Zamora-Ros (2013)	.88625842	.82667404	.9501375
Combined	.91789765	.8712695	.96702124

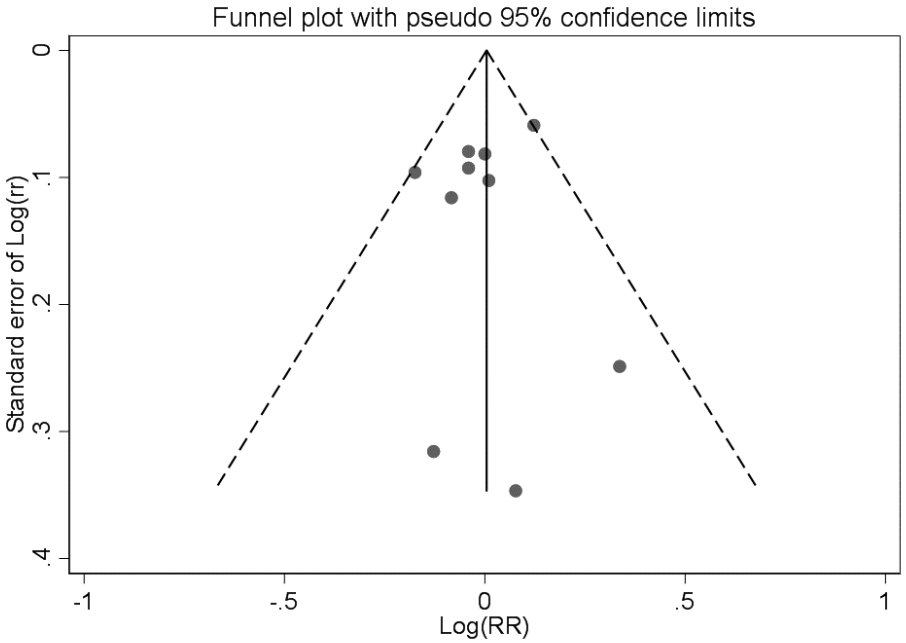
Supplemental Figure 1b. Funnel plot and sensitivity analysis for anthocyanin intake and risk of CVD mortality.

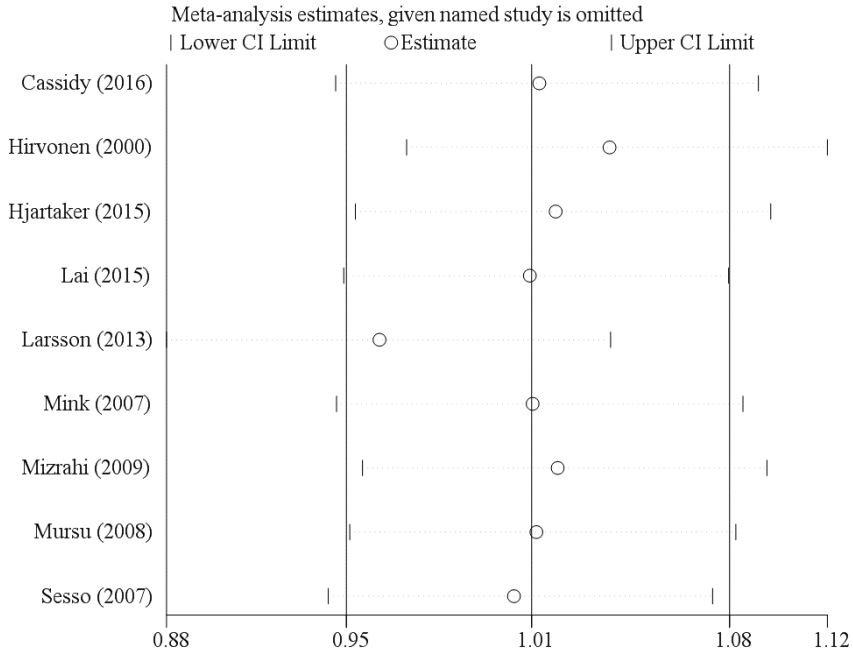




Study omitted	Estimate	[95% Conf.	Interval]
Cassidy (2013)	.99013674	.89431351	1.096227
Cassidy (2016)	.88034195	.65913522	1.175786
Sesso (2007)	.9406572	.85209078	1.0384291
Combined	.95939624	.87033911	1.0575661

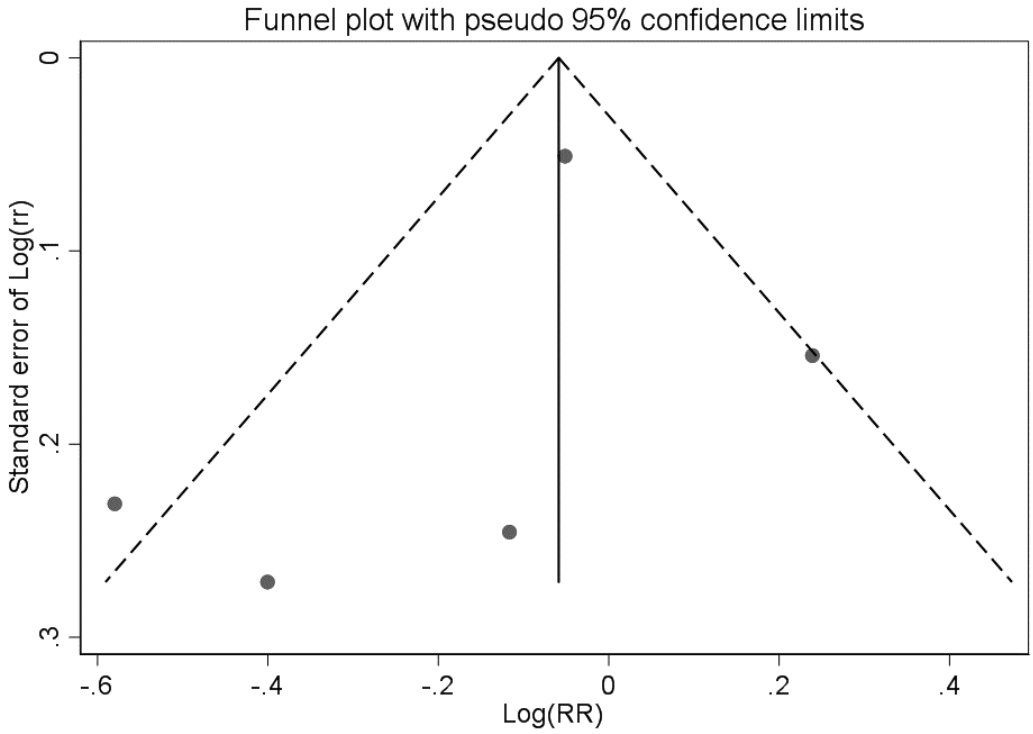
Supplemental Figure 1c. Funnel plot and sensitivity analysis for anthocyanin intake and risk of MI.

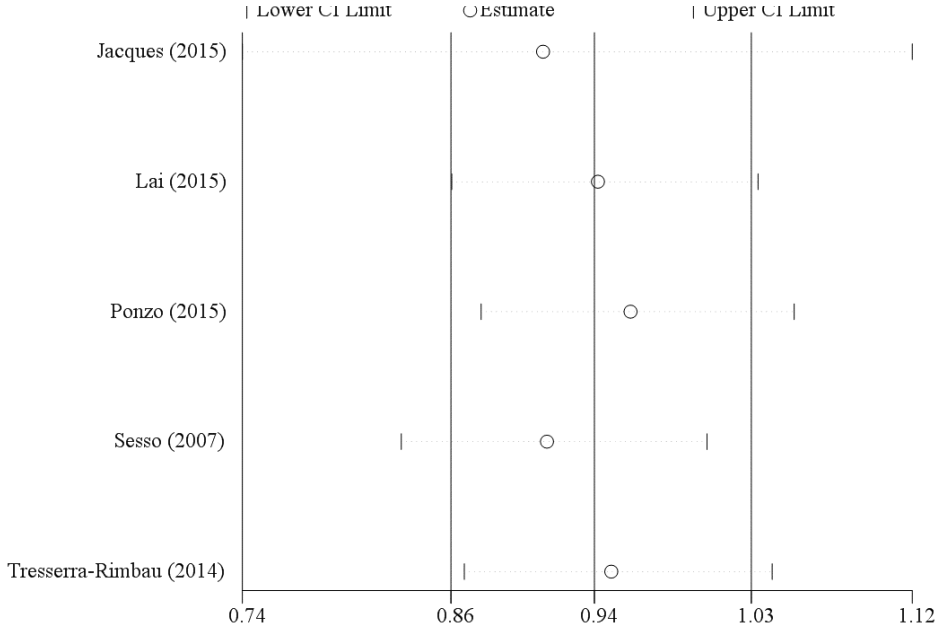




Study omitted	Estimate	[95% Conf.	Interval]
Cassidy (2016)	1.015512	.94383711	1.0926298
Hirvonen (2000)	1.0402271	.96879983	1.1169205
Hjartaker (2015)	1.0212653	.95076901	1.0969887
Lai (2015)	1.0121735	.94670784	1.0821663
Larsson (2013)	.95925242	.88426882	1.0405945
Mink (2007)	1.0131505	.94415277	1.0871903
Mizrahi (2009)	1.0219747	.95326972	1.0956314
Mursu (2008)	1.0144709	.94879335	1.0846949
Sesso (2007)	1.0065974	.94120324	1.0765352
Combined	1.0128037	.94760188	1.0824918

Supplemental Figure 1d. Funnel plot and sensitivity analysis for anthocyanin intake and risk of Stroke.





Study omitted	Estimate	[95% Conf.	Interval]
Jacques (2015)	.9139924	.74424934	1.1224492
Lai (2015)	.9449861	.8624633	1.0354049
Ponzo (2015)	.96336401	.87904626	1.0557694
Sesso (2007)	.91621375	.83399618	1.0065366
Tresserra-Rimbau (2014)	.95251501	.86959332	1.0433438
Combined	.94301674	.86204753	1.0315911

Supplemental Figure 1e. Funnel plot and sensitivity analysis for anthocyanin intake and risk of total CVD.