An overview and update on the epidemiology of flavonoid intake and cardiovascular disease risk: Flavonoid intake and cardiovascular disease risk


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An overview and update on the epidemiology of flavonoid intake and cardiovascular disease risk

Short title: Flavonoid intake and cardiovascular disease risk

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Abstract

There is an accumulating body of literature reporting on dietary flavonoid intake and the risk of cardiovascular disease (CVD) in prospective cohort studies. This makes apparent the need for an overview and update on the current state of the science. To date, at least 27 prospective cohorts (in 44 publications) have evaluated the association between estimated habitual flavonoid intake and CVD risk. At this time, the totality of evidence suggests long-term consumption of flavonoid-rich foods may be associated with a lower risk of fatal and non-fatal ischemic heart disease (IHD), cerebrovascular disease, and total CVD; disease outcomes which are principally, though not exclusively, composed of cases of atherosclerotic CVD (ASCVD).

To date, few studies have investigated outcome specific ASCVD, such as peripheral artery disease (PAD) or ischemic stroke. Of the flavonoid subclasses investigated, evidence more often implicates diets rich in anthocyanins, flavan-3-ols, and flavonols in lowering the risk of CVD. Although inferences are restricted by confounding and other inherent limitations of observational studies, causality appears possible based on biological plausibility, temporality, and the relative consistency of the reported associations. However, whether the associations observed represent a benefit of the isolated bioactives per se, or are a signal of the bioactives acting in concert with the co-occurring nutrient matrix within flavonoid-bearing foods, are issues of consideration. Thus, the simple interpretation, and the one most relevant for dietary advice, is that consumption of flavonoid-rich foods or diets higher in flavonoids, appear nutritionally beneficial in the prevention of CVD.
## Table of contents

1. Introduction ....................................................................................................................... 5
2. Dietary occurrence and intake of flavonoids ................................................................. 6
3. Calculation of flavonoid intake and how this has changed over the years ..................... 10
4. Biological mechanisms of flavonoids in cardiovascular disease ................................. 15
5. Flavonoid intake and cardiovascular disease risk: epidemiological evidence .............. 17
   a. Total cardiovascular disease ..................................................................................... 17
   b. Ischemic heart disease ............................................................................................. 27
   c. Cerebrovascular disease .......................................................................................... 36
   e. Peripheral artery disease ......................................................................................... 44
6. Discussion: current evidence—future epidemiological frontiers ................................. 47
7. Conclusion ...................................................................................................................... 54
1. Introduction

The 2017 Global Burden of Disease study observed that nutrition is a major contributor to cardiovascular morbidity and mortality, both in developed and developing countries. This highlights the need to shift global dietary trends for the health of humanity. Overall dietary patterns, generally higher in fruits, vegetables, wholegrains, nuts, seeds, legumes, seafood and yoghurt; moderate to modest in poultry, milk, cheese, eggs, alcohol and unprocessed red meat; and low to absent in processed meat, refined grains, and ultra-processed foods; have been identified as providing cardio-metabolic benefits, which translate into a lower risk of developing cardiovascular disease (CVD). The contribution of other regularly consumed items, such as tea and coffee, to cardio-metabolic health appears less certain. Attempts to determine the contribution of specific nutritive components, underpinning the benefits of such dietary patterns rich in plant foods, is underway. In addition to vitamins, minerals, and macronutrients, plant foods contain a wide variety of bioactive agents, such as (poly)phenols, terpenoids, and nitrogen containing compounds that might confer health benefits. Flavonoids, a class of (poly)phenolics, have gained specific interest as findings from epidemiological studies throughout the 1990’s and 2000’s indicate they may be responsible, at least in part, for the cardiovascular-protective benefits of diets rich in plant foods.

In 1993, the first prospective study to evaluate the cardio-protective benefits of flavonoids observed that participants with the highest flavonoid intakes had a lower risk of ischemic heart disease (IHD) mortality. Since this time, a rapidly accumulating body of epidemiological evidence, supported by growing evidence from human intervention studies, has emerged. Comprehensively reviewed in 2005 by Arts and Hollman, as well as by Graf et al., and again in 2012 by Peterson et al., we have sought to provide an overview and update on the epidemiological evidence regarding the association between habitual flavonoid intake and CVD incidence and mortality in prospective cohorts. We begin by covering the dietary occurrence and intake of flavonoids. First, we describe the different flavonoid subclasses and...
their food sources before continuing with an overview of global consumption trends and intake differences between populations. The quantification of flavonoid intake follows, describing advances in composition databases, how these have changed over the years, and the potential impact this has for cohort studies. An overview of biological mechanisms, by which flavonoids mitigate CVD initiation and progression, precedes the synthesis of epidemiological evidence regarding the association of flavonoid intake with CVD risk. Here, we focus on total flavonoid intake and consumption of major flavonoid subclasses and their association with total CVD and CVD of atherosclerotic origin, before discussing major findings from outcome-specific prospective cohort studies on IHD, cerebrovascular disease and peripheral artery disease (PAD) spanning 1993 to March 2020. Following the update on the current state of the art, the discussion addresses broader issues of importance to flavonoid epidemiology research. We discuss the central hypothesis, its evidence and limitations, that flavonoid-rich dietary intake mediates intermediate endpoints of CVD, which modulates the trajectory of chronic CVD. Thereafter, we highlight critical gaps and needs for advancing flavonoid epidemiology, including the use of objective intake measures, cumulative measures of exposure, and the rationale for targeting ASCVD rather than all-cause CVD. Concluding the review is a summary of progress in the field over the past ~25 years and major future pathways for its continued development.

2. Dietary occurrence and intake of flavonoids

Flavonoids are the most common group of plant phenolics occurring ubiquitously throughout food plants and their products. To this end, a recent report estimates over 15,000 different flavonoids have been identified throughout the plant Kingdom, with at least several hundred occurring in edible components. In plants, flavonoids occur as secondary metabolites, which are not essential to the growth or life of the plant. Instead, flavonoids contribute to the protection of plants against pests and herbivores, as well as plant disease, season and climate,
geography, and other local challenges, to increase the plant’s overall ability to survive.\textsuperscript{12,13} Flavonoids, found in edible plants and their food products, are part of the human diet.\textsuperscript{13,14} They are not essential constituents in the classic nutrition sense; rather, they are non-nutrients, which can exert biologically relevant effects \textit{in vivo}.\textsuperscript{13,14} Based on their underlying structure, flavonoids can be subdivided into six major subclasses, namely, the flavonols, flavones, flavanones, isoflavones, flavan-3-ols, and anthocyanins.\textsuperscript{15} Collectively, dietary sources are quite diverse and range from nuts, seeds, wholegrains, and legumes to fruits, herbs, and vegetables, and also include oils, tea, wine, and beer.

In foods, the flavonols, flavones, flavanones, isoflavones and anthocyanins occur mostly as glycosides (i.e., attached to a sugar moiety) rather than as aglycones (i.e., as free form compounds).\textsuperscript{16,17} Flavan-3-ol monomers on the other hand, are generally not present as glycosides; instead they occur most often in free form or as gallic acid esters.\textsuperscript{16,17} As the glycosidic flavonoids may be linked with many different sugars,\textsuperscript{17,18} to simplify analyses, these flavonoids are often quantified and reported as aglycone equivalents (abbreviated herein as AE). This is achieved by hydrolysing the flavonoid from their attached sugars and measuring the total content of the flavonoid aglycone present in a single food.\textsuperscript{17–19} Gallic acid esters of flavan-3-ols however, are often reported as such, without any conversion to aglycones; in turn, these values are often incorporated in total flavonoid intake calculations, while other subclasses are included as AE.\textsuperscript{19,20} When quantified exclusively as AE, it becomes apparent that only several dozen flavonoids are relatively common among foods and dietary intake.\textsuperscript{13,21} For example, in the French observational study \textsc{Su}plémentation on \textsc{Vi}tamines et \textsc{Mi}néraux \textsc{AntioXydants} (\textsc{Su}.	extsc{Vi}.	extsc{Max}), across the 4,942 participants, >180 different flavonoids were consumed by the cohort as part of their normal dietary intake, of which, 80\% occurred as glycosides; the remainder occurring as aglycones or esters.\textsuperscript{21} Yet, when quantified entirely as AE, only 47 aglycones were commonly consumed.\textsuperscript{21}
While the flavonoid subclass and compound make-up of plant foods differs within and between their genus, across species (e.g., apple varieties), the flavonoid subclasses present are more or less consistent. However, the absolute concentration of individual flavonoids in foods is strongly influenced by their growing, storage, and processing conditions. The flavonoids are the most widely occurring of the flavonoids, being found in almost all fruits and vegetables.

The richest flavonol sources include onions (>20 mg/100 g AE) and spinach (>10 mg/100 g AE); although, black tea (>3 mg/100 ml AE) and apples (>2 mg/100 g AE with skin), are usually the more important dietary sources, as they are generally consumed in greater quantities. Anthocyanins are uniquely concentrated in berries (e.g., blueberries typically contain >100 mg/100 g AE) however, are present in a several other foods (e.g., plum >40 to >500 mg/100 g AE depending on species; kidney beans cooked >3 mg/100 g AE). On the other hand, flavones are found in very limited quantities throughout foods. Examples of flavone food sources include whole grain wheat flour (e.g., sorghum flour >2 mg/100 g AE), orange juice (>5 mg/100 ml AE) and extra virgin olive oil (>1 mg/100 ml AE). Flavanones are mostly concentrated in citrus fruits (e.g., orange >40 mg/100 g AE) and their juices. Isoflavones are found in trace quantities throughout vegetables and grains; their only rich sources include soy bean products (e.g., edamame >40 mg/100 g AE). Lastly is the flavan-3-ol subclass, which consist of several sub-groups including monomers (also termed catechins), as well as oligomers and polymers (also termed proanthocyanidins). Flavan-3-ols are also found in black tea in various oxidized oligomeric and polymeric forms, such as theaflavins and thearubigins, formed during tea leaf fermentation. Catechins and proanthocyanidins are present in high concentrations in dark chocolate (>200 mg/100 g), apples (>100 mg/100 g), red wine (>50 mg/100 ml), nuts (e.g., almonds >150 mg/100 g) and berries (e.g., blueberry >300 mg/100 g) while, black tea is uniquely high in monomers, theaflavins and thearubigins (>100 mg/100 ml). However, the measurement of thearubigins is often inaccurate, and thus, they are often not included in intake estimates.
Despite contributing most tea flavan-3-ols.\textsuperscript{20} As an aside, coffee is not a rich source of flavonoids (<0.5 mg/100 ml AE total flavonoids), however, it is abundant in phenolic acids.\textsuperscript{17} Ultimately, an individual’s flavonoid exposure is dependent on their routine diet.

Globally, the average intake of total flavonoids is estimated at \(~400\) mg/day (calculated principally as AE though, not including thearubigins).\textsuperscript{28} However, dietary patterns and regional food consumption differs greatly between countries and continents so there can be large variability in the daily intake of flavonoids across the globe.\textsuperscript{28} While limitations exist in diet data collection methods and the estimation of flavonoid intake,\textsuperscript{13} some general estimates can be made. Yet, to preface these estimates, researchers tend to broadly estimate total flavonoid intake in terms of AE, however, this includes free form flavan-3-ols and sometimes esterified flavan-3-ols as well. In the United States of America, the mean intake of total flavonoids varies from \(~250\) to \(~400\) mg/day AE (including thearubigins) while, in the United Kingdom, the average intake is reported to be \(>1000\) mg/day AE (including thearubigins), which is more similar to intakes reported in Australia AE (\(~650\) to \(~700\) mg/day including thearubigins).\textsuperscript{13,28} The differences likely reflect a high intake of black tea in the latter two countries where black tea may provide 60 to 80\% of total flavonoid intake.\textsuperscript{13,28} Across mainland Europe, in Mediterranean countries (defined here as Greece, Spain, Italy, and Southern France), the mean intake of total flavonoids is \(~250\) to \(~400\) mg/day AE (not including thearubigins); the major contributor being fruit.\textsuperscript{28} In Nordic countries, such as Finland, average total flavonoid intake is \(~200\) to \(~250\) mg/day AE (not including thearubigins) while, to the Far East in China, \(~225\) mg/day AE (not including thearubigins) is generally consumed; the major contributor being tea,\textsuperscript{28} of which, green tea is a popular variety.\textsuperscript{29}

As total daily flavonoid intake varies across the globe, so too does the consumption of flavonoid subclasses and their respective individual compound make-up. That being, in general, the flavan-3-ols are the most commonly consumed subclass, typically contributing
upwards of ~80% towards total flavonoid intake, followed by flavanones (~8–10%), anthocyanins (~7–10%), flavonols (~7–9%), flavones (~1–2%), and isoflavones which, at the population level, are generally (except in Asia) consumed in trace quantities.\textsuperscript{28} Yet, even within populations, differences in flavonoid intake occurs among subgroups. For example, in the European Prospective Investigation into Cancer, across Europe, higher flavonoid intake was associated with increasing age, education and physical activity as well as being female and a health-food consumer.\textsuperscript{30} Thus, across geographic regions and socio-demographic characteristics, differences in both the quantity and proportion of flavonoid-rich foods consumed results in a wide variability of flavonoid exposure among populations.\textsuperscript{28}

3. Calculation of flavonoid intake and how this has changed over the years

Flavonoid intake in nutritional epidemiology has historically been estimated from food frequency questionnaires (FFQ) and diet histories using food composition tables. By the mid-1970’s, emerging composition data, detailing a small range of foods, were used to calculate the first estimate of daily flavonoid intake from dietary origin.\textsuperscript{31,32} However, advances in analytical methods by the early 1990’s enabled a more accurate measure of the flavonoid content of foods and up-dated composition tables were published by Hertog \textit{et al.} in 1992 and 1993.\textsuperscript{18,33,34} These tables provide information on AEs of two flavones (i.e., luteolin and apigenin) and three flavonols (i.e., quercetin, myricetin and kaempferol) across ~60 foods and beverages.\textsuperscript{18,33,34} The food composition tables by Hertog \textit{et al.} were used to estimate flavonoid intake in the Zutphen Elderly cohort in order to examine the association between flavonoid intake and CVD risk—the first prospective cohort study with this aim.\textsuperscript{5} This prompted an interest in assessing associations between habitual flavonoid consumption and CVD health outcomes and the data on flavonoid intakes developed by Hertog \textit{et al.} were used as the cornerstone to estimate flavonoid intake in several prospective studies on CVD throughout the later 90’s and early 2000’s.\textsuperscript{35–42} Interest in flavonoids other than flavones or flavonols was building, yet, at the turn
of the century (1999 to 2000), accurate data on the flavan-3-ol content of foods was largely unavailable. At this time, Arts et al. measured the content of six flavan-3-ol monomers found in >60 foods and beverages. This spurred the investigation of flavan-3-ol intake and CVD risk in several prospective cohorts. However, a database detailing the gamut of flavonoids was lacking and studies were starting to integrate several smaller reports on the flavonoid composition of foods into their analyses.

In 2003 the United States Department of Agriculture (USDA) released their database on the flavonoid content of 225 foods and beverages covering 26 of the most commonly occurring flavonoids (expressed as AE, except for flavan-3-ols which were reported as free form aglycones and esters without conversion) throughout five subclasses (i.e., flavonols, flavones, flavanones, anthocyanins and flavan-3-ols (including catechins, theaflavins and thearubigins)). Around the same time, they also released their database on the isoflavone content of 128 foods covering three aglycones (i.e., daidzein, genistein, and glycitein) and in 2004 they released their database on the proanthocyanidin content of 205 foods. Following their initial release, the USDA databases have undergone several major updates. In 2007, the flavonoids database was updated to include 392 foods (Release 2.0), and in 2011, again expanded to include 500 foods (Release 3.0), although no other monomeric compounds were incorporated. In 2008, the isoflavone database was updated to contain 557 foods (Release 2.0), and in 2015 the proanthocyanidin database (Release 2.0) was updated to contain 283 foods; again neither incorporating further compounds. In 2014 the USDA released their extended database, which covers ~2900 foods and six major flavonoid subclasses except proanthocyanidins. Since their initial release, the USDA databases have been used regularly for estimating flavonoid intake in prospective cohort studies. However, different flavonoid glycosides are known to vary in their biological activity and a database detailing the range of flavonoids naturally occurring in foods was thus developed.
The Phenol-Explorer database was released online in 2009. This database differs to the USDA databases in that it contains information on individual flavonoids as they are found in food (i.e., mainly as glycosides). Phenol-Explorer also contains information on other (poly)phenol classes, such as stilbenes, lignans and phenolic acids in addition to several other subclasses (e.g., curcuminoids), collectively providing information on 502 (poly)phenols in 452 foods. With regards to flavonoids, Phenol-Explorer compiles data that was (largely) measured utilising chromatography without hydrolysis (in which each individual flavonoid form, as found in food, is quantified) while, the USDA databases (are generally) compiled from analysis utilising chromatography after hydrolysis (which removes the glycosides enabling total quantification of the aglycones). To obtain AE using Phenol-Explorer, the user converts the glycoside or ester via molecular weight conversion into total aglycones. Alternatively, Phenol-Explorer also contains content values as measured by chromatography after hydrolysis, but, these data have been seldom used in cohort studies.

The first release of Phenol-Explorer contained information on >270 unique flavonoids. Importantly, unlike the USDA database, Phenol-Explorer does not include data on thearubigins however, both contain information on theaflavins. Phenol-Explorer was updated in 2012 (version 2.0) and again in 2013 (version 3.0) to contain information on flavonoid pharmacokinetics as well as the effect of food processing on flavonoid retention throughout food preparation. Like the USDA databases, Phenol-Explorer has been widely used to estimate flavonoid intake in epidemiological studies. Of note, Knaze et al. used Phenol-Explorer to calculate the flavonoid content of foods and meals consumed in the European Prospective Investigation into Cancer, using retention factors, they generated the (poly)phenol composition of 19,899 raw and prepared, generic and multi-ingredient food items—the database of which is accessible online. While other databases detailing the flavonoid content of foods exist (e.g., EuroFIR-BASIS), these have been much less frequently incorporated in epidemiological research.
While there has been substantial advances in the completeness of flavonoid databases, such food composition tables are known to be limited by their coverage of food items and nutrients within foods. In addition, the flavonoid composition of foods is variable as they are dependent on their variety, growing, storage, and processing or cooking conditions; thus such data are generally regarded as approximations of the contents of food. To this end, limited attention has been paid to the potential differences between flavonoid databases and the impact this may have on flavonoid intake estimates. In a recent method-comparison study, Ivey et al. studied the congruence between Phenol-Explorer (version 2.1) and both the USDA database for estimating flavonoid (Release 3.1) and proanthocyanidin (Release 1.0) consumption. From FFQs, flavonoid intake was estimated in AE, using all databases. The USDA and Phenol-Explorer databases were highly correlated for total flavonoid intake and all subclasses ($r > 0.8$, $P < 0.001$) except flavones ($r = 0.34$, $P < 0.001$). However, the absolute mean intake values were significantly different between databases across all subclasses and total flavonoids. Despite these differences, when participants were classified as low, moderate and high consumers as estimated using both databases, in general, participants fell into the same intake categories. Thus, in epidemiology research, these issues need to be considered in systematic reviews and meta-analyses of data.

Regardless of the database used to calculate flavonoid intake, estimates can only be as accurate as the assessment tools used to quantify habitual diet. The quantification of habitual intake is complex and difficult because individuals eat a wide variety of different foods, in different combinations, in different amounts, in different frequencies. Until recent years, only a few main methods of assessing dietary intake have been available: the food diary, diet history, 24-hr recall and FFQ. Of these, the FFQ is the most efficient and therefore most widely used. However, most FFQs used in prospective cohort investigations to date were constructed prior to the development of the USDA flavonoid databases and were not specifically designed for the measurement of flavonoid intake. As such, they may not
optimally reflect the variability in flavonoid intake, due to the omission of some flavonoid-bearing foods or the group of similar food items within the same question (e.g., berries), making it impossible to discern which specific food type/s the individual eats. Performance and validation studies of FFQs, specifically in regards to flavonoid intake, have seldom been conducted; while an FFQ may have been validated for other nutrients or food groups, this does not necessarily imply that it can reasonably assess intake of flavonoids. In this context, the extrapolation of absolute values from FFQs as accurate measures of intake is not usually justified. However, they may still provide a rank of usual consumption, allowing researchers to roughly separate out irregular (lower) vs regular (higher) consumers, in large samples, given the FFQ contains ample questions on major flavonoid bearing items. Ultimately, inaccurate dietary assessment biases results towards the null as noise in the data increases; the implications are that true associations are likely stronger than those reported. In more recent years, validation studies of FFQs for flavonoid intake have begun to emerge, however, it is hoped that nutrition science will look to shift to emerging technologies, such as biomarkers or smartphone methods, which may allow for a more robust assessment of dietary intake in the future.

As described above, with advances in both knowledge and technology, methods used to estimate flavonoid intake in cohorts with data on habitual diet have changed over the last ~25 years (Figure 1). Prior to 2003 and the development of the USDA database and later, Phenol-Explorer, total flavonoid intake reported in cohort studies (~3 to 25 mg/day) was calculated from the estimated intake of 5 to 10 compounds spanning 2 to 4 subclasses (flavonols, flavones, flavan-3-ols monomers and flavanones). This underestimation of flavonoid intake (and conceivable misclassification of participants) is a critical limitation of these early studies often not recognized in the literature. More recent studies present results for 5 to 7 flavonoid subclasses and have an average flavonoid intake ranging from approximately 130 to 700 mg/day. Further, flavonoid intake values between studies may not be directly comparable as the studies used different dietary assessment tools that were not necessarily
validated for measuring flavonoids. These differences in calculating flavonoid intake must be kept in mind; comparing findings from studies prior to development of the USDA and Phenol-Explorer databases, to findings from studies post their release, is inherently difficult. In this review, we refer to a study as estimating total flavonoid intake, only if the estimate is a composite of ≥5 subclasses; often, studies do not include isoflavones in their composite scores due to their low dietary occurrence in most populations. Where a study has estimated the sum of ≤4 subclasses, we describe the sum of subclasses (e.g., intake of flavones plus flavonols etc.).

[Insert Figure 1]

Figure 1. Timeline of development of landmark flavonoid-food composition databases and the cumulative number of prospective cohort publications reporting on the intake of flavonoid subclasses and/or total consumption and cardiovascular disease risk. USDA, United States Department of Agriculture.

4. Biological mechanisms of flavonoids in cardiovascular disease

Following their digestion and absorption, flavonoid metabolites enter the blood stream where they may interact with molecular mechanisms mediating CVD. Over 170 biological effects have been reported for flavonoids and flavonoid-rich foods, reviewed recently by Williamson et al. Regarding their CVD-protective actions, the most recognized activity of flavonoids is their positive effects on the vasculature. In randomised controlled trials (RCTs), flavonoids and flavonoid-rich foods have been shown to lower blood pressure, improve endothelial function, and augment arterial stiffness. Most evidence from RCTs is for the flavan-3-ol subclass, which, in most populations, is the greatest contributor to total flavonoid intake.

Meta-analyses of human RCTs have shown beneficial effects of flavonoids on major CVD risk factors. Blood pressure is one well-established risk factor for CVD. A meta-analysis of 91
RCTs comparing the effect of a flavan-3-ol intervention with a control on blood pressure showed a significant decrease in SBP (−1.46 mm Hg, 95% CI: −2.27, −0.65 mm Hg) and DBP (−0.99 mm Hg, 95% CI: −1.50, −0.45 mm Hg). Another independent risk factor of CVD, endothelial dysfunction (impaired nitric oxide-mediated vasodilation), predicts atherothrombotic clinical events. Aside from impaired vasodilation, a dysfunctional endothelium may be permeable to atherogenic lipoproteins and secrete vascular adhesion molecules; critical initial steps in the development of an atherosclerotic lesion. A meta-analysis comparing the effects of flavan-3-ols with controls showed a significant increase in acute FMD response measured from 1 to 6 h (1.70%, 95% CI: 1.31%, 2.08%; 24 RCTs) and a significant improvement in chronic FMD measured after a few weeks of intervention (1.21%, 95% CI: 0.70%, 1.73%; 23 RCTs). Additionally, several RCTs have reported a decrease in arterial stiffness after a flavonoid-based intervention, independent of blood pressure changes. The exact mechanisms behind these improvements are yet to be established but may include enhanced nitric oxide bioavailability, increased expression of heme oxygenase-1, and inhibition of angiotensin-converting enzyme activity. Quantifying the effect of flavonoids on atherosclerosis itself in RCTs is inherently difficult due to slow disease initiation and progression. However, flavonoid interventions have been reported to lower LDL-cholesterol and triglycerides, and increase HDL-cholesterol in human studies, while animal and in vitro studies provide evidence that flavonoids improve inflammatory-status via the interference of pro-oxidant enzyme-signalling cascades or adhesion molecule expression. Unfortunately, fluctuations in background cytokine production and difficulty in detecting subtle changes in inflammatory status make this very difficult to assess in human intervention studies; a meta-analysis of RCTs on inflammatory biomarkers found no difference between flavan-3-ols and controls. Thus, in the absence of long-term clinical trials allowing for the long preclinical phase or quantifiable progression of
atherosclerosis, observational prospective cohort studies provide us with the strongest evidence for the protective role of habitual flavonoid intakes in the manifestation of CVD.

5. Flavonoid intake and cardiovascular disease risk: epidemiological evidence

a. Total cardiovascular disease

Disease overview. Currently, the global burden of CVD accounts for approximately one third of all deaths and is the leading cause of mortality and disability worldwide.93,94 Total CVD is an umbrella term for a broad range of diseases of the heart and circulatory system, of which, the main underlying pathology is atherosclerosis.95,96 The major clinical manifestations of atherosclerotic CVD (ASCVD) include IHD, PAD, and ischemic stroke.97 Other CVDs, not aetiologically linked to atherosclerosis, include: structural heart disease, congenital heart disease, rheumatic heart disease, arrhythmias, and other disease of the arterial and venous system.98 In 2015, the Global Burden of Disease study estimated the worldwide prevalence of CVD for the most common causes of CVD death.94 They found there was ~422.7 million prevalent cases of CVD worldwide, of which, ASCVD was the main contributor with ~154.7 million cases of PAD (~36.6%), ~110.5 million cases of IHD (~26.2%) and ~24.9 million cases of ischemic stroke (~5.9%); the other ~31.3% of prevalent cases constituted haemorrhagic and other strokes, hypertensive heart disease, cardiomyopathy, atrial fibrillation, endocarditis and other cardiovascular and circulatory diseases.94 In the same year, ~17.9 million people died from CVD, wherein, it is estimated that ~66% died from ischemic stroke and IHD.94 In high-income countries, such as within the continent of North America, the proportion of the population dying of IHD and ischemic stroke, reaches as high as ~75% of total CVD.94 A range of in vitro and in vivo studies suggest that flavonoids exert beneficial effects on the arterial vascular system suggesting that CVDs of atherosclerotic origin may be particularly amenable to a flavonoid intervention.79,99,100 While the bulk of existing literature on flavonoid intake and
risk of total CVD includes CVDs of both atherosclerotic and non-atherosclerotic origins, as highlighted above, cases are dominated by ASCVD.

Cohort characteristics. To date, at least 19 prospective cohorts (in 21 publications) have estimated flavonoid intake and the risk of total CVD incidence and/or mortality (Table 1). Nearly all studies reporting on CVD incidence defined this as the first non-fatal or fatal event. However, one study restricted outcomes to first time events resulting in hospitalisation, while another defined incidence as the first non-fatal event only. Around half of the studies defined total CVD as any type of CVD, mostly using the International Classification of Diseases (ICD) 9th Revision, codes 390–459, and their equivalent ICD-10 codes I00–I99 to identify case events (n = 10 publications). However, all such studies reported on morality outcomes—which is dominated by ASCVD (Table 1). At the same time, studies reporting on total CVD incidence, tend to restrict analysis to events of cerebrovascular disease and IHD, with some further incorporating death from any CVD (Table 1). Thus, they too are principally reporting on ASCVD. However, given this outcome definition, studies on CVD incidence tend to overlook non-fatal events of PAD; although PAD is the most prevalent ASCVD, a large proportion of PAD goes undiagnosed and therefore, in a cohort study, PAD usually represents proportionally less events. Other studies exclusively analysed events of ASCVD, while two studies did not provide complete outcome definitions. At least one study presented analyses of total CVD as per several outcome definitions, of which we report the most relevant for this review. Effectively, while the definition of total CVD is somewhat heterogeneous, the cases are primarily, but not exclusively, ASCVD-related.

Other cohort characteristics were also heterogeneous. The cohorts vary in size (from 774 to 98,469 participants) as well as their follow-up timeframe (from 4.3 to 25 years) and location (United States of America (n = 5), Australia (n = 2), Asia (n = 2), and Europe (n = 10)). Most cohorts recruited middle-aged participants (e.g., ~50 years), while some recruited older
individuals (e.g., 65+ years). Habitual diet has been mainly assessed using FFQs ($n = 14$ cohorts) and flavonoid intake mostly estimated using the USDA ($n = 9$ publications) and/or Phenol-Explorer ($n = 6$ publications) databases (Table 1). Only one study indicated flavonoid intake was estimated as glycosides (and other naturally occurring structures); all others appear to report intake as AE (including free form and potentially esterified flavan-3-ols).

**Total flavonoids.** As previously mentioned, in this review, we discuss a study as assessing total flavonoid intake, only if this estimate was a composite of ≥5 subclasses. The association between such total flavonoid intake and total CVD incidence has been studied in at least five cohorts, while at least nine cohorts report on total CVD mortality. In 2017, Grosso *et al.* meta-analysed five of these cohorts reporting on total CVD mortality ($n = 137,629$, with $5,351$ CVD deaths), and found a trend towards a lower risk of death with higher total flavonoid intake (multivariable adjusted risk estimate for highest vs lowest intake category: 0.83, 95% CI: 0.68, 1.03). While high heterogeneity was observed among these studies ($I^2 = 67\%$), a subsequent sensitivity analysis (by systematic exclusion of cohorts) reduced heterogeneity ($I^2 = 20\%, P = 0.29$) and highlighted a significant association between total flavonoid intake and CVD mortality ($n = 4$ cohorts, $n = 136,566$, $n = 5,273$ deaths; multivariable adjusted risk estimate for highest vs lowest intake category: 0.88, 95% CI: 0.79, 0.98). The excluded study by Ivey *et al.* reported the strongest association of all studies (multivariable adjusted risk estimate for highest vs lowest intake category: 0.32, 95% CI: 0.16, 0.61). Ivey *et al.* undertook analysis in the oldest cohort in the meta-analysis ($n = 1,063$, mean age: $80 \pm 3$ years at baseline) with ~30% of the sample experiencing pre-existing CVD at baseline, which may explain why such a strong association was observed. That is, participants would have been at very high risk of dying of CVD (7.3% died of CVD). The Grosso *et al.* meta-analysis found no evidence of publication bias, which was supported by a similar meta-analysis published in the same year. However, this finding may not be overly convincing as both meta-analyses included a relatively small number of cohorts. Grosso *et al.* also found an association, albeit non-significant,
between CVD mortality and total flavonoid intake when the meta-analysis was confined exclusively to studies adjusting for dietary confounders in addition to sociodemographic factors (n = 3 studies; multivariable adjusted risk estimate for highest vs lowest intake category: 0.71, 95% CI: 0.33, 1.51; $I^2 = 79\%$). Consequently, cofounding from unmeasured nutritional components may have biased results, although conclusions are limited due to a small sample size and wide confidence intervals. Since the Grosso et al. meta-analysis, a further three studies on total flavonoid intake and CVD mortality have been published with results trending towards a lower associated risk of CVD mortality with higher flavonoid intake. Unlike for total CVD mortality, a meta-analysis on total flavonoid intake and CVD incidence has not been published. However, significant inverse relationships emerge in three of the five cohorts reporting on this outcome. While other meta-analyses in the field have pooled heterogeneous flavonoid exposures and outcome measures, overall their findings suggest that flavonoid-rich dietary patterns are associated with a lower risk of CVD events.

Flavonoid subclasses. The impact of individual flavonoid subclasses on total CVD risk has been studied across incidence (n = 9 cohorts) and mortality (n = 14 cohorts) outcomes. The results typically report an inverse association reaching statistical significance for all subclasses in at least one or more cohorts (Table 1). Grosso et al. meta-analysed several of the cohorts reporting on CVD mortality. A significantly lower risk of death was found for the highest compared to the lowest intake categories of anthocyanins (n = 5 cohorts; RR = 0.89, 95% CI: 0.83, 0.95; $I^2 = 0\%$), catechins (termed flavan-3-ols in their manuscript; n = 7 cohorts; RR = 0.82, 95% CI: 0.71, 0.95; $I^2 = 53\%$), proanthocyanidins (n = 4 cohorts; RR = 0.89, 95% CI: 0.81, 0.97; $I^2 = 0\%$), flavonols (n = 6 cohorts; RR = 0.79, 95% CI: 0.63, 0.99; $I^2 = 67\%$), flavones (n = 5; RR = 0.85, 95% CI: 0.75, 0.96; $I^2 = 25\%$), and flavanones (n = 5 cohorts; RR = 0.84, 95% CI: 0.73, 0.96; $I^2 = 34\%$) but not isoflavones (n = 6 cohorts; RR = 1.01, 95% CI: 0.94, 1.08; $I^2 = 0\%$) in multivariable adjusted models. The high heterogeneity observed for flavonols and catechins was reportedly due to a study by Mursu et al., which after removal,
reduced heterogeneity without any material impact on the results. These findings were in agreement with two other recent meta-analyses on CVD mortality risk and intake of anthocyanins and flavan-3-ols. Since these meta-analyses, at least three further prospective cohorts have been published on each subclass (except isoflavones); results typically trending towards a beneficial association, though, reaching significance only for anthocyanin intake and lower CVD mortality risk in one cohort (Table 1). While less research has focused on CVD incidence, it is suspected that outcomes would echo CVD mortality findings.

**Dose-response.** The intake of flavonoids associated with significant benefit has been the subject of investigation. In a dose-response meta-analysis, Grosso et al. report a 4% lower risk of CVD mortality for every ~100 mg/day increment in total flavonoid intake (n = 5 cohorts, n = 137,629, total CVD deaths = 5,351; multivariable adjusted risk estimate: 0.96, 95% CI: 0.92, 1.00; \( I^2 = 68\% \)). While a significant linear relationship emerged over the range of intakes studied (\( P_{\text{linearity}} < 0.001 \)) it is unlikely that the true relationship maintains linearity indefinitely. It is more plausible that there is a plateau in the trend somewhere, where further consumption affords no added benefit, although the point of plateau likely differs in populations with different underlying risk. Nonetheless, it appears that only moderate intakes may be needed to positively affect health outcomes, and that higher intakes likely afford no added benefit. Results supporting this assertion can be seen in analyses from the Cancer Prevention Study II Nutrition Cohort (n = 98,469), and the Danish Diet Cancer and Health Cohort (n = 56,048), both of which show nonlinear dose-response thresholds, somewhere around ~250 to ~500 mg/day AE of total flavonoid intake. However, extrapolation of absolute values from FFQs is not usually possible unless a study has validation data.

**Effect modification.** As indicated, the beneficial associations of dietary patterns high in flavonoids might differ, in different population subgroups. It has previously been hypothesized that the association between flavonoid intake and CVD mortality may be stronger in persons...
at a higher risk of CVD. Early findings of a study by Mink et al. weakly supported this hypothesis. In their analysis, significant inverse associations between flavanone intake (but not other subclasses or total intake) and CVD mortality were found in ever-smokers, but not never-smokers following multivariable adjustment for age and energy. Bonfondo et al., then showed a lower risk of all-cause mortality (31% were CVD-related) for higher flavonoid intakes only amongst participants with at least one early mortality risk factor (smoking, high alcohol consumption, no regular exercise or obesity). Following on from this, more results by Bonfondo et al. show clear effect modification by smoking status and alcohol intake at baseline although, not for sex, physical activity, or diabetes following multivariable adjustment for lifestyle factors. That is, the inverse association between total flavonoid intake and total CVD mortality appeared to differ in smokers in contrast to non-smokers as well as in high alcohol over low alcohol consumers suggesting dietary flavonoid intake may partially mitigate harmful effects of such activities (although, the smoking and high alcohol intake groups would remain at higher overall risk of CVD compared to their counterparts). Given this finding, it stands to reason that associations between flavonoid intake and CVD may appear to be stronger in cohorts that have a higher proportion of smokers and high alcohol consumers. It is also plausible that further unidentified effect modifiers exist, such as in racial or ethnic populations, which warrants further investigation. However, as “at risk” populations have a higher underlying risk of CVD, these findings require investigation on an absolute, as well as, a relative scale.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Baseline age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate</th>
<th>Analysis</th>
<th>Exposure†</th>
<th>Intake (low vs high) mg/dy</th>
<th>Total CVD incidence (95% CI)</th>
<th>% for trend</th>
<th>Total CVD mortality (95% CI)</th>
<th>% for trend</th>
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<td><strong>2019</strong></td>
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<tr>
<td>Bondonno</td>
<td>Denmark</td>
<td>56,048</td>
<td>56</td>
<td>23</td>
<td>ICD-10</td>
<td>FFQ/PE</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: &lt;9.6 vs ≥33.2</td>
<td>178 (0.53, 1.29)</td>
<td>2.794 (0.80, 1.29)</td>
<td>0.97 (0.83, 1.17)</td>
<td>0.85 (0.55, 1.29)</td>
<td>1.10 (0.69, 1.77)</td>
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<td></td>
<td>(Danish Diet, Cancer &amp; Health study)</td>
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<tr>
<td>Bondonno</td>
<td>Australia</td>
<td>2,349</td>
<td>65</td>
<td>14</td>
<td>ICD-9:40–414 &amp; 430–438</td>
<td>FFQ/USDA ratio by tertiles</td>
<td>Hazard ratio by tertiles</td>
<td>Anthocyanidin: &lt;3.8 vs ≥9.2</td>
<td>213 (0.55, 1.18)</td>
<td>1.80 (0.51, 3.0)</td>
<td>0.94 (0.46, 1.03)</td>
<td>0.92 (0.46, 1.96)</td>
<td>0.94 (0.53, 1.98)</td>
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<td>(Blue Mountains Eye Study)</td>
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<tr>
<td>Dalgaard</td>
<td>Denmark</td>
<td>53,552</td>
<td>56</td>
<td>23</td>
<td>Hospital admission for atherosclerotic CVD, ICD-10 I20: I25, I63, I70–74</td>
<td>FFQ/PE ratio by quintiles</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: &lt;9 vs ≥53</td>
<td>1,773 (0.95, 1.09)</td>
<td>3.981 (0.90, 1.20)</td>
<td>0.89 (0.67, 1.18)</td>
<td>0.80 (0.56, 1.15)</td>
<td>0.83 (0.53, 1.29)</td>
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<td>(Danish Diet, Cancer &amp; Health study)</td>
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<td>ICD-9:345</td>
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<tr>
<td>Mendonca</td>
<td>Spain</td>
<td>17,065</td>
<td>37</td>
<td>10</td>
<td>Stroke, MI, death from CVD causes</td>
<td>FFQ/PE ratio by quintiles</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: &lt;27.5 vs ≥55.0</td>
<td>113 (0.53, 0.98)</td>
<td>0.94 (0.68, 1.34)</td>
<td>0.90 (0.82, 1.04)</td>
<td>0.80 (0.49, 1.35)</td>
<td>0.83 (0.53, 1.29)</td>
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<td>(SUN cohort)</td>
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<td>Adiouch</td>
<td>France</td>
<td>84,158</td>
<td>44</td>
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<td>Stroke, MI, acute coronary syndrome, or angioplasty</td>
<td>FFQ/PE ratio by tertiles</td>
<td>Hazard ratio by tertiles</td>
<td>Anthocyanidin: &lt;3 vs ≥17</td>
<td>602 (0.55, 0.85)</td>
<td>0.06 (0.05, 0.07)</td>
<td>0.76 (0.62, 0.94)</td>
<td>0.67 (0.53, 0.85)</td>
<td>0.72 (0.56, 0.94)</td>
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<td>(Nursing-Santé)</td>
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<td>Ivey</td>
<td>USA</td>
<td>93,145</td>
<td>36</td>
<td>18</td>
<td>ICD-8:390–458 &amp; ICD-9:390–459</td>
<td>FFQ/USDA ratio by quintiles</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: &lt;3 vs ≥17</td>
<td>213 (0.51, 1.18)</td>
<td>2.794 (0.80, 1.29)</td>
<td>0.97 (0.83, 1.17)</td>
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<td>(NHS II)</td>
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<td>Author, year</td>
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<td>Baseline age (yrs)</td>
<td>Follow-up (yrs)</td>
<td>Outcome assessment</td>
<td>Flavonoid intake estimate</td>
<td>Analysis</td>
<td>Intake (low vs high mg/day)</td>
<td>Total CVD incidence a Fully adjusted estimate (95% CI) [high vs low]</td>
<td>P for trend</td>
<td>Total CVD mortality a Fully adjusted estimate (95% CI) [high vs low]</td>
<td>P for trend</td>
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<td>Dower 2016</td>
<td>Netherlands (Zutphen) 774 (M) to 84 65 25 ICD-9 390-459 &amp; 798.2 Diet history/Arts et al. 12,16 Catechin &lt;9.4 vs ≥19.5  —  —  — 329 0.79 (0.58, 1.08) —</td>
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<td>Ivey 2015</td>
<td>Australia (CAIFOS) 1,063 (post-menopause women) &gt;75 5 ICD-9 390-459 &amp; ICD-10 100-199 FFQ/PE and USDA Catechin &lt;547 vs ≥813 — — 78 0.34 (0.17, 0.69) —</td>
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<td>Jacques 2015</td>
<td>USA (Framingham Offspring Cohort) 2,880 (M &amp; F) 54 14.9 Stroke, TIA, CHD, heart failure or peripheral vascular disease FFQ/PE and USDA Relative risk for each 2.5-fold ↑ in daily intake Anthocyanin: 0.95 (0.86, 1.05)† 0.30  —  —  —</td>
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<td>Pouzo 2015</td>
<td>Italy (no cohort name) 1,658 (M &amp; F) 45 to 64 12 Mortality: ICD-9 390-459 &amp; 798.1; no codes specified for incidence FFQ/PE and USDA Hazard ratio by quintiles Anthocyanin: 0.56 (0.36, 0.89)* 0.26 84 0.67 (0.38, 1.18) —</td>
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<td>Vogiatzoglou 2015</td>
<td>UK (EPIC) M: 11,252 (F: 13,633) 40 to 75 11.1 ICD-9 410-448 &amp; ICD-10 110-119 7-day diary Flavonol Database Hazard ratio by quintiles Male: Total flavan-3-ol: k=198 vs k=2008 4,403 0.90 (0.81, 0.99) 0.176 1,154 0.95 (0.74, 1.20) 0.607 Female: Total flavan-3-ol: k=179 vs k=1828 4,060 1.01 (0.91, 1.13) 0.364 833 0.81 (0.60, 1.09) 0.166</td>
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<td>Talaei 2014</td>
<td>Singapore (Singapore Chinese Health Study) 63,257 (M &amp; F) 45 to 74 14.7 ICD-9 390-459 FFQ/PE and Flavonol Database, Hazard ratio by quartile Female: Total flavan-3-ol: k=54 vs k=56.1 4,780 1.00 (0.91, 1.10) 0.83</td>
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<td>Treserra- Rimbhu 2014</td>
<td>Spain (PREDEMID) 7,172 (M &amp; F) 67 4.3 MI, stroke or death from CVD causes, Fatty Flavonol Database, Hazard ratio by quintiles Anthocyanin: 11.8 vs 74.6 273 0.67 (0.39, 1.13) 0.05  —  —  —</td>
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Table 1. Continued: Summary of prospective cohort studies investigating the association of flavonoid intake with total CVD incidence and mortality

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Baseline age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate</th>
<th>Analysis</th>
<th>Exposure</th>
<th>Intake (low vs high) mg/day</th>
<th>Total CVD incidence</th>
<th>Total CVD mortality</th>
<th>$P$ for trend</th>
<th>$P$ for trend</th>
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</thead>
<tbody>
<tr>
<td>Ivey 2015†</td>
<td>Australia (CAIFOS)</td>
<td>1,063 (post-menopause women) 1,041</td>
<td>&gt;75</td>
<td>5</td>
<td>ICD-9 410–414, 428–433, 438 &amp; 440–444</td>
<td>FFQ</td>
<td>Odds ratio by quintile</td>
<td>Anthocyanidin: &lt;23 vs &gt;41 0.35 (0.14, 0.83) 0.01</td>
<td>—</td>
<td>—</td>
<td>64</td>
<td>0.67 (0.33, 1.34) 0.35</td>
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<td>Zamora-Ros 2013</td>
<td>Spain (EPIC)</td>
<td>40,622 (M &amp; F)</td>
<td>29 to 69</td>
<td>13.6</td>
<td>ICD-9 &amp; ICD-10 (no codes specified) Diet history/Flavonoid intake</td>
<td>Diet history/</td>
<td>Hazard ratio for each 2-fold increase in intake</td>
<td>Anthocyanidin: &lt;24 vs &gt;35 0.56 (0.29, 1.12) 0.20</td>
<td>—</td>
<td>—</td>
<td>416</td>
<td>0.96 (0.88, 1.03)† —</td>
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<tr>
<td>Mc-Cullough 2012</td>
<td>USA (Cancer Prevention Study II)</td>
<td>99,469 (M &amp; F)</td>
<td>&gt;69</td>
<td>7</td>
<td>ICD-9 &amp; ICD-10 I20–I99</td>
<td>FFQ</td>
<td>Rate</td>
<td>Anthocyanidin: &lt;5.5 vs ≥16.7 0.88 (0.76, 0.97) 0.04</td>
<td>—</td>
<td>—</td>
<td>2,771</td>
<td>0.86 (0.76, 0.97) 0.04</td>
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<tr>
<td>Murau 2008</td>
<td>Finland (Kuopio IHD study)</td>
<td>1,950 (M)</td>
<td>42 to 60</td>
<td>15.2</td>
<td>ICD-9 390–459 &amp; ICD-10 I00–I99 4-day diary/USDA</td>
<td>FFQ</td>
<td>Relative risk by quintiles</td>
<td>Anthocyanidin: &lt;121.5 vs ≥359.7 0.82 (0.73, 0.92) 0.01</td>
<td>—</td>
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<td>102</td>
<td>0.99 (0.62, 1.55) 0.193</td>
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<tr>
<td>Kokubo 2007</td>
<td>Japan (Japan Public Health Cohort I)</td>
<td>40,462 (M &amp; F + post-menopause sub-group)</td>
<td>40 to 59</td>
<td>12</td>
<td>ICD-10 I21–I23, I46, I60–I61, I63 &amp; I693</td>
<td>FFQ</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: &lt;16.2 vs ≥37.7 0.87 (0.29, 2.52) 0.103</td>
<td>—</td>
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<td>57</td>
<td>0.87 (0.29, 2.52) 0.103</td>
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</table>
Table 1. Continued: Summary of prospective cohort studies investigating the association of flavonoid intake with total CVD incidence and mortality

<table>
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<th>Baseline age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Analysis</th>
<th>Exposure&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Intake (low vs high) mg/day</th>
<th>Fully adjusted estimate (95% CI)</th>
<th>P for trend</th>
<th>Fully adjusted estimate (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mink 2007&lt;sup&gt;23&lt;/sup&gt;</td>
<td>USA (Iowa WHS) (post-menopause women)</td>
<td>34,489</td>
<td>55 to 69</td>
<td>16</td>
<td>ICD-9 FFQ&lt;sup&gt;2&lt;/sup&gt;</td>
<td>USDA Rate ratio by quintiles</td>
<td>Anthocyanidin: &lt;0 vs &gt;0.01</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2,316</td>
<td>0.91 (0.83, 0.99)</td>
<td>0.032</td>
</tr>
<tr>
<td>van der Schoor 2005&lt;sup&gt;30&lt;/sup&gt;</td>
<td>Netherlands (Prospect-EPIC)</td>
<td>16,165 (F) to 70</td>
<td>49</td>
<td>6.25</td>
<td>ICD-9 FFQ/Self-developed database</td>
<td>Hazard ratio by quartiles</td>
<td>Isoflavone: —</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>518</td>
<td>0.97 (0.74, 1.27)</td>
<td>—</td>
</tr>
<tr>
<td>Sesso 2003&lt;sup&gt;31&lt;/sup&gt;</td>
<td>USA (WHS)</td>
<td>38,445 (F)</td>
<td>&gt;45</td>
<td>6.9</td>
<td>MI, heart bypass, PTCA stroke &amp; CVD death</td>
<td>FFQ/Mostly</td>
<td>Relative risk by quintiles</td>
<td>Flavone + flavonol: x̄±8.8 vs x̄±47.4</td>
<td>729</td>
<td>0.88 (0.68, 1.14)</td>
<td>0.63</td>
<td>1.05 (0.62, 1.78)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Diet data capture method/Major flavonoid database used.  
* Subclass composition and calculations of flavonoid intake may differ between studies; the term ‘Total’ is used to denote the sum of 5 or more flavonoid subclasses.  
* Incidence is defined as the first fatal or non-fatal event unless otherwise indicated.  
* The model with the most covariate adjustments in each manuscript is reported; covariates differ between manuscripts.  
* Includes non-fatal events only.  
* Flavonoid intake estimated as found in food (i.e., mostly glycosides).  
* Represents the association for a proportional difference in flavonoid intake (see corresponding analysis description).  
* Abbreviations: CAIFOS, Calcium Intake Fracture Outcome Age Related Extension Study; CHD, coronary heart disease; CVD, cardiovascular disease; EPIC, European Prospective Investigation into Cancer and Nutrition; F, female; FFQ, food frequency questionnaire; ICD, International Classification of Disease; IHD, ischemic heart disease; M, male; MI, myocardial infarction; NHS, Nurses’ Health Study; ODC, other derived compounds (e.g. theaflavins and/or thearubigins); PA, proanthocyanidin; PE, Phenol-Explorer; PREDIMED, Prevención con Dieta Mediterránea; PTCA, percutaneous transluminal coronary angioplasty; SUN, Seguimiento Universidad de Navarra; TIA, transient ischemic attack; USA, United States of America; USDA, United States Department of Agriculture; UK, United Kingdom; WHS, Women’s Health Study.  
* x̄, mean; x̄ median.
b. Ischemic heart disease

Disease overview. Globally, IHD is the main cause of death accounting for ~9 million fatalities in 2015. Collectively, IHD is the term for a group of clinical syndromes characterized by myocardial ischemia, most often due to atherosclerosis. Atherosclerosis can cause narrowing of the coronary arteries (stenosis) which can lead to angina pectoris, marked by paroxysms of chest pain. Secondly, atherosclerosis can cause thrombus formation in the coronary arteries, interrupting the blood supply to the heart and causing necrosis of the myocardium, in addition to several other manifestations such as coronary aneurysms. A growing body of mechanistic research suggests flavonoids could lower the risk of IHD by mitigating atherosclerosis and its risk factors such as hypertension, hypercholesterolemia, and endothelial dysfunction. Thus, the following describes findings from studies examining the association of flavonoid intake with IHD (also referred to as coronary heart disease) or its distinct disease sequelae such as myocardial infarction.

Cohort characteristics. Since 1993, at least 21 prospective cohorts (in 28 publications) have reported on flavonoid intake and the risk of IHD incidence and/or mortality (Table 2). As an aside, one other prospective study has reported on flavonoids and IHD risk, but it had an ecological study design, which differs from the other studies and is thus, not presented in Table 2. Of the publications presented, some report on the same study over different years of follow-up. For example, the Zutphen Elderly Study reports on flavone and flavonol intake and IHD risk at five and 10 years of follow-up, as well as catechin intake and IHD risk after 10 and 25 years of exposure. Other cohorts report on different classes of flavonoid intake across multiple papers, reflecting the timeline of development of several key flavonoid databases. For example, the Iowa Women’s Health Study first reported on IHD risk and intake of flavones and flavonols in 1999 using tables by Hertog et al. to estimate consumption, then in 2001 on catechin intake using tables by Arts et al. to estimate exposure, then again in 2007.
using the USDA database to estimate all flavonoid subclasses and total intake; in each instance, they used updated follow-up time frames.\(^{39,46,50}\)

Of the cohorts investigating flavonoid intake and IHD to date, most were conducted in Europe \((n = 10)\) and the United States of America \((n = 8)\) with only a minority being conducted in other parts of the world \((n = 1)\) and Asia \((n = 2)\). A total of 18 studies report on IHD mortality while, 19 report on IHD incidence (with nine reporting on both incidence and mortality). Nearly all studies reporting on IHD incidence defined this as the first non-fatal or fatal event. However, incidence was occasionally defined as the first non-fatal event only.\(^{40,76}\)

Some authors presented results using both definitions (of which we present the former in Table 2).\(^{42}\) Approximately half of the studies reporting on incidence restricted analysis to case events of myocardial infarction.\(^{5,40–42,45,56,76,101,118,119}\) Studies reporting on IHD mortality tended to identify events more broadly using ICD-9 codes 410–414, or their equivalent. Some authors presented results for total IHD as well as selected IHD subtypes, for which we present the results of the broader definition in Table 2.\(^{37}\) Most cohorts recruited middle aged adults, although they varied in their demographic characteristics as well as sample size \((from \ 755 \ to \ 98,469 \ participants)\) and follow-up periods \((from \ 4.9 \ to \ 28 \ years)\). Habitual diet was mainly assessed using FFQs \((n = 17 \ publications)\) and the source used to estimate flavonoid exposure varied from the tables by Hertog et al. \((n = 10 \ publications)\) to the USDA databases \((n = 7 \ publications)\), tables by Arts et al. \((n = 3 \ publications)\), Phenol-Explorer \((n = 2 \ publications)\), the Singapore Composition Database \((n = 1 \ publication)\), and several other methods \((n = 5)\).

All studies appear to report intake as AEs \((including \ free \ form \ and \ potentially \ esterified \ flavan-3-ols)\), except for one cohort, which reports flavonoids expressed as glycosides \((and \ their \ other \ naturally \ occurring \ structures)\).\(^{60}\)

Total flavonoids. The association between total flavonoid intake \((estimated \ as \ \geq 5 \ subclasses)\) and IHD incidence has been studied in at least four cohorts while mortality rates have been
studied in at least three. Of these studies, significant findings were only observed in the Danish Diet, Cancer & Health study which examined first time events of hospitalisation for IHD (multivariable adjusted hazard ratio for highest vs lowest intake category: 0.91, 95% CI: 0.85, 0.98). This study had a large sample size (n = 53,552), high number of cases (n = 5,323) and long follow-up period (23 years). While no meta-analyses have focused exclusively on total flavonoid intake and IHD, a 2017 meta-analysis pooled heterogeneous flavonoid exposures and examined their association with IHD mortality. Here, Liu et al. describe a trend towards a lower risk of IHD death with higher flavonoid intake (n = 4 cohorts; multivariable adjusted risk estimate for highest vs lowest intake category: 0.74, 95% CI: 0.54, 1.02; I² = 61%). However, these results differ to a prior meta-analysis by Jiang et al., who also pooled 4 cohorts reporting on flavonoid intake and IHD death, yet a clearly lowered risk with higher flavonoid exposure was reported (multivariable adjusted risk estimate for highest vs lowest intake category: 0.82, 95% CI: 0.74, 0.90; I² = 22%). The reasons for these disparities are not clear, although may have arisen from study selection criteria. Jiang et al. further pool 15 cohorts reporting on IHD mortality (n = 4), IHD incidence (n = 10) and total CVD mortality (n = 1), examining any flavonoid exposure, including total intake (n = 3), single subclass estimates (n = 5) or other subclass combinations (n = 7). In this analysis, a significantly lower risk of IHD events for persons with a higher flavonoid intake was observed (n = 452,564; n = 7,233 cases; multivariable adjusted risk estimate for highest vs lowest intake category: 0.85, 95% CI: 0.79, 0.91, I² = 26%). Thus, while the body of evidence lends support to a beneficial association between higher flavonoid intake and IHD risk, additional research to further the granularity of evidence may be beneficial.

Flavonoid subclasses. The association between individual flavonoid subclasses and IHD incidence has been studied in at least 16 cohorts (in 19 publications), while mortality events have been studied in at least 11 cohorts (across 18 publications). Across cohorts, a lower risk of IHD tends to emerge, reaching significance in at least one or more studies, but not in more
than half, for each individual subclass, except isoflavones for which no significant associations have been observed (Table 2). In 2003, Huxley and Neil pooled seven cohorts reporting on intakes of flavonols and risk of IHD mortality (although, some of the included studies additionally included flavones in their intake estimates). The authors observed a significantly lower risk of IHD mortality with higher exposures ($n = 105,737$; multivariable adjusted risk estimate for highest vs lowest intake category: 0.80, 95% CI: 0.69, 0.93). More recently, Kimble et al. pooled studies reporting on intakes of anthocyanins (or berries as surrogate indicators of intake) and both IHD incidence and mortality. Overall, this analysis observed an association in favour of a lower risk with higher intakes and minimal publication bias was apparent ($n = 5$ studies, $n = 58,638$; multivariable adjusted risk estimate for highest vs lowest intake category: 0.91, 95% CI: 0.83, 0.99; $I^2 = 12.0\%$). Kimble et al. also restricted the meta-analysis to events of myocardial infarction (including incidence and mortality), yet observed no association with anthocyanin/berry intake ($n = 3$ studies, $n = 175,656$; multivariable adjusted risk estimate for highest vs lowest intake category: 1.00, 95% CI: 0.68, 1.46, $I^2 = 78.1\%$). Raman et al. reported a meta-analysis of two studies on IHD incidence and total flavan-3-ol intake finding an inverse association ($n = 41,563$; multivariable adjusted risk estimate for highest vs lowest intake category: 0.81, 95% CI: 0.66, 0.99; $I^2 = 60.8\%$) although, no association was for observed monomer exposure ($n = 2$ studies, $n = 44,514$; multivariable adjusted risk estimate for highest vs lowest intake category: RR = 1.00 95% CI: 0.76, 1.32).

Seemingly, across subclasses, evidence appears stronger for anthocyanin, flavanol and flavan-3-ol intake in lowering risk of IHD.

**Effect modification.** Modification of the relationship between flavonoid intake and IHD has been explored in a limited number of studies. Goetz et al. explored effect modification of flavonoids on IHD by age, sex, race (non-Hispanic white and black Americans), educational attainment, smoking, and physical activity in the REasons for Geographic and Racial Differences in Stroke (REGARDS) study. The authors enrolled 16,678 black and white males
and females (≥45 yrs old, 42% black, 55% female) living throughout the United States of America. After 6.0 ± 1.9 years of follow-up, 589 IHD incidences occurred however, the effect of flavonoids did not vary by the tested factors; confirmation of these findings in a larger sample would be of interest. Cassidy et al. examined the associations between anthocyanin intake and incidence of myocardial infarction among strata of age (participants <65 or ≥65 years old), the presence of type 2 diabetes mellitus and hypertension, in the Health Professionals Follow-Up Study (n = 43,880). After 24 years of following the all-male cohort, they found no effect modification by age at baseline, nor diabetes. However the inverse association between anthocyanin intake and myocardial infarction was stronger in participants who were not hypertensive (HR: 0.81; 95% CI: 0.69, 0.96) compared to participants diagnosed with hypertension (HR: 1.05; 95% CI: 0.79, 1.39; P Interaction = 0.03). Cassidy et al. suggest this may have occurred because medication use may have exceeded the capacity for flavonoids to lower the risk of CVD. In the Nurses' Health Study II (n = 93,600; all females), Cassidy et al. also identified risk factors that may modify the relationship between flavonoid intake and myocardial infarction. They tested for effect modification by age, smoking, physical activity, prevalent hypertension, type 2 diabetes mellitus, alcohol intake, and body mass index. No significant interactions emerged.
### Table 2. Summary of prospective cohort studies investigating the association of flavonoid intake with ischemic heart disease

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Baseline age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate*</th>
<th>Analysis</th>
<th>Exposure‡</th>
<th>Intake (low vs high) mg/day</th>
<th>Fully adjusted estimate (95% CI)</th>
<th>P for trend</th>
<th>Fully adjusted mortality (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalgaard 2019</td>
<td>Denmark (Danish Diet, Cancer &amp; Health study)</td>
<td>53,352 (M &amp; F)</td>
<td>56</td>
<td>23</td>
<td>Hospital admission for ICD-10 I20- I25</td>
<td>FFQ/</td>
<td>Quintile</td>
<td>Total: k=174 vs 1000</td>
<td>5,323</td>
<td>0.91 (0.85, 0.98)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adrissou 2018</td>
<td>France (Nuninet- Santé)</td>
<td>84,159 (M &amp; F)</td>
<td>44.1</td>
<td>4.9</td>
<td>MI, acute coronary syndromes, &amp; angioplasty</td>
<td>3 x 24 hr diet record/ PE***</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanin: Catechin: Theaflavin: PA: Flavonone: Flavone: Flavonol: Isoflavone:</td>
<td>309</td>
<td>0.74 (0.53, 1.03)</td>
<td>0.07</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bondanorno 2018</td>
<td>Australia (Blue Mountains Eye Study)</td>
<td>2,349 (M &amp; F)</td>
<td>64.7</td>
<td>14</td>
<td>ICD-9 410–414 &amp; ICD-10 I21–I25.9</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: Catechin + ODC: PA: Flavonone: Flavone: Flavonol: Isoflavone: CHD</td>
<td>4,046</td>
<td>0.97 (0.87, 1.07)</td>
<td>0.44</td>
<td>1,816</td>
<td>1.10 (0.94, 1.28)</td>
</tr>
<tr>
<td>Cassidy 2016</td>
<td>USA (Health Professionals Follow-Up Netherlands (Zutphen))</td>
<td>43,880 (M)</td>
<td>32</td>
<td>24</td>
<td>MI</td>
<td>FFQ/ Mostly USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: Flavonol:</td>
<td>4,046</td>
<td>0.98 (0.88, 1.08)</td>
<td>0.87</td>
<td>0.95 (0.81, 1.11)</td>
<td>0.22</td>
</tr>
<tr>
<td>Dower 2016</td>
<td>USA (REGARDS)</td>
<td>774 (M)</td>
<td>65</td>
<td>25</td>
<td>ICD-9 410–414 &amp; 792.2</td>
<td>Diet history/ Arts et al.*</td>
<td>Hazard ratio by quintiles</td>
<td>Catechin:</td>
<td>—</td>
<td>—</td>
<td>0.74 (0.47, 1.16)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Goetz 2016</td>
<td>USA (REGARDS)</td>
<td>16,678 (M &amp; F)</td>
<td>≥45</td>
<td>6</td>
<td>MI &amp; CHD death</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: Catechin + ODC: PA: Flavonone: Flavonol: CHD</td>
<td>589</td>
<td>0.73 (0.55, 0.96)</td>
<td>0.05</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Jacques 2015</td>
<td>USA (Framingham Offspring Cohort)</td>
<td>2,880 (M &amp; F)</td>
<td>54</td>
<td>14.9</td>
<td>MI, angina pectoris, coronary insufficiency, and CHD death</td>
<td>FFQ/USDA</td>
<td>Relative risk for each 2.5- fold ↑ in daily intake</td>
<td>Anthocyanidin: Catechin: PA + ODC: Flavonone: Flavone: Flavonol:</td>
<td>261</td>
<td>0.98 (0.86, 1.12)</td>
<td>0.76</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Flavonoid intake is estimated using a FFQ or diet record.

‡Exposure is defined as flavonoid intake in quintiles.

The table includes studies that investigated the association of flavonoid intake with ischemic heart disease (IHD), with outcomes such as incidence and mortality. The studies were conducted in various countries, including Denmark, France, Australia, USA, and the Netherlands, with different sample sizes and follow-up durations. The flavonoid intake was estimated using FFQs or diet records, and the analyses were performed using quintiles or tertiles of intake. The results are presented as fully adjusted estimates with 95% confidence intervals (CI) and P-values for trend. The studies also varied in terms of baseline demographics and outcomes, such as hospital admission for MI, acute coronary syndromes, and angioplasty in the Dalgaard study, and various cardiovascular outcomes in the other studies.
### Table 2. Continued: Summary of prospective cohort studies investigating the association of flavonoid intake with ischemic heart disease

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Base-line age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate</th>
<th>Analysis</th>
<th>Exposure</th>
<th>Intake (low vs high) mg/day</th>
<th>Fully adjusted estimate (95% CI) [low vs high]</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vogiatzo-oglua 2015</td>
<td>USA</td>
<td>98,469</td>
<td>-69</td>
<td>7</td>
<td>ICD-9</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>Total flavan-3-ol:</td>
<td>5.4 vs 36.1</td>
<td>2,335 0.88 (0.77, 1.01)</td>
</tr>
<tr>
<td>Talaei 2014</td>
<td>Singapore (Singapore Chinese Health Study)</td>
<td>63,257 &amp; 698</td>
<td>45</td>
<td>14.7</td>
<td>ICD-9</td>
<td>Flavonol Flavonol Food Database</td>
<td>Hazard ratio by quintiles</td>
<td>Females:</td>
<td>Total flavan-3-ol:</td>
<td>5.4 vs 36.1</td>
<td>1,325 0.96 (0.80, 1.17)</td>
</tr>
<tr>
<td>Cassidy 2013</td>
<td>USA (NHS II)</td>
<td>93,600</td>
<td>25</td>
<td>18</td>
<td>M &amp; F</td>
<td>FFQ/Singapore Composition Database</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>190 vs 350</td>
<td>2,697 0.97 (0.83, 1.16)</td>
<td>0.41</td>
</tr>
<tr>
<td>Mo- Cullough 2012</td>
<td>USA (Cancer Prevention Study II)</td>
<td>40,462</td>
<td>40</td>
<td>12</td>
<td>ICD-9</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>Total flavan-3-ol:</td>
<td>2.6 vs 28.4</td>
<td>1,286 0.79 (0.67, 0.94)</td>
</tr>
<tr>
<td>Kokubo 2007</td>
<td>Japan (Japan PHS Cohort I)</td>
<td>40,462</td>
<td>40</td>
<td>12</td>
<td>ICD-9</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>Total flavan-3-ol:</td>
<td>2.5 vs 27.7</td>
<td>0.87 (0.74, 1.04)</td>
</tr>
<tr>
<td>Lin 2007</td>
<td>USA (NHS)</td>
<td>66,360</td>
<td>30</td>
<td>12</td>
<td>Incidence: MI Mortality: ICD -9</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>Total flavan-3-ol:</td>
<td>16.2 vs 37.7</td>
<td>66 0.37 (0.14, 0.98)</td>
</tr>
<tr>
<td>Mink 2007</td>
<td>USA (Iowa WHS)</td>
<td>34,498</td>
<td>55</td>
<td>16</td>
<td>ICD-9</td>
<td>FFQ/USDA</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>Total flavan-3-ol:</td>
<td>9.0 vs 29.6</td>
<td>924 0.77 (0.47, 1.29)</td>
</tr>
<tr>
<td>Marniemi 2005</td>
<td>Finland (no study name)</td>
<td>755</td>
<td>65</td>
<td>10</td>
<td>ICD-9</td>
<td>Diet history/ Nutrica database</td>
<td>Hazard ratio by quintiles</td>
<td>Males:</td>
<td>Total flavan-3-ol:</td>
<td>9.0 vs 29.6</td>
<td>1,329 0.88 (0.78, 0.99)</td>
</tr>
</tbody>
</table>

**Note:** The table continues with more data on the association of flavonoid intake with ischemic heart disease, including details on study methodology, outcomes, and statistical results.
Table 2. Continued: Summary of prospective cohort studies investigating the association of flavonoid intake with ischemic heart disease

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Base-line age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate</th>
<th>Analysis</th>
<th>Intake (low vs high) mg/day</th>
<th>Exposure</th>
<th>n</th>
<th>IHD incidence*</th>
<th>P for trend</th>
<th>n</th>
<th>IHD mortality</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>van der Schoor 2003</td>
<td>Netherlands (Prospect EPIC)</td>
<td>16,165 (F)</td>
<td>49 to 70</td>
<td>6.25</td>
<td>ICD-9 410-414, 427.5</td>
<td>FFQ/self-developed database</td>
<td>Hazard ratio by quartiles</td>
<td>Isoflavone: —</td>
<td>372</td>
<td>0.94 (0.68, 1.30)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Sesso 2003</td>
<td>USA (WHS)</td>
<td>38,445 (F)</td>
<td>&gt;45</td>
<td>6.9</td>
<td>MI using WHO criteria</td>
<td>FFQ/Hertog et al.</td>
<td>Relative risk by quintiles</td>
<td>Flavone + flavonol: ≤8.8 vs ≤47.4</td>
<td>—</td>
<td>0.82 (0.51, 1.33)</td>
<td>0.89</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Knekt 2002</td>
<td>Finland (Finnish Mobile Clinic)</td>
<td>9,131 (M &amp; F)</td>
<td>54 to 84</td>
<td>28</td>
<td>ICD-8 410-414</td>
<td>Diet history/ Mostly local analysis</td>
<td>Relative risk by quartiles</td>
<td>Flavone + flavonol: ≤44.8 vs &gt;44.8</td>
<td>—</td>
<td>0.76 (0.49, 1.18)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Geleijnse 2002</td>
<td>Netherlands (Rotterdam Study)</td>
<td>4,807 (M &amp; F)</td>
<td>≥55</td>
<td>5.6</td>
<td>ICD-10 I21</td>
<td>Diet history/ Mostly local analysis</td>
<td>Relative risk by tertiles</td>
<td>Flavone: ≤16.8 vs &gt;40.0</td>
<td>146</td>
<td>0.76 (0.49, 1.18)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Arts 2001</td>
<td>Netherlands (Zutphen)</td>
<td>806 (M) to 84</td>
<td>65</td>
<td>10</td>
<td>Incidence: MI Mortality: ICD 9 410-414 &amp; 429.2</td>
<td>Diet history/ Mostly local analysis</td>
<td>Risk ratio by tertiles</td>
<td>Catechin: ≤49.0 vs &gt;85.9</td>
<td>90</td>
<td>0.49 (0.27, 0.88)</td>
<td>0.017</td>
<td>90</td>
<td>0.70 (0.39, 1.26)</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td>Arts 2001</td>
<td>USA (Iowa WHS)</td>
<td>34,492 (F)</td>
<td>55 to 69</td>
<td>13</td>
<td>ICD-9 410-414 &amp; 429.2</td>
<td>FFQ/Mostly local analysis</td>
<td>Risk ratio by quintiles</td>
<td>Catechin: ≤3.7 vs &gt;52.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>767</td>
<td>0.85 (0.67, 1.07)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Hirvonen 2001</td>
<td>Finland (ATBC Study)</td>
<td>25,372 (M smokers)</td>
<td>50 to 69</td>
<td>6.1</td>
<td>Incidence: MI Mortality: ICD 9 410-414 &amp; 429.2</td>
<td>Diet history/ Mostly local analysis</td>
<td>Relative risk by quartiles</td>
<td>Flavone + flavonol: ≤3.9 vs &gt;17.8</td>
<td>1,122</td>
<td>0.77 (0.64, 0.93)*</td>
<td>0.81</td>
<td>0.89 (0.71, 1.11)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Yochum 1999</td>
<td>USA (Iowa WHS)</td>
<td>34,492 (post-menopause women)</td>
<td>~61</td>
<td>10</td>
<td>ICD-9 410-414 &amp; 429.2</td>
<td>FFQ/Mostly local analysis</td>
<td>Relative risk by quintiles</td>
<td>Flavone + flavonol: ≤5.7 vs ≥18.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>438</td>
<td>0.62 (0.44, 0.87)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Hertog 1997</td>
<td>Netherlands (Zutphen)</td>
<td>804 (M)</td>
<td>65 to 84</td>
<td>10</td>
<td>Incidence: MI Mortality: ICD 9 410-414 &amp; 429.2</td>
<td>Diet history/ Mostly local analysis</td>
<td>Relative risk by tertiles</td>
<td>Flavone + flavonol: ≤19 vs &gt;29.9</td>
<td>92</td>
<td>0.62 (0.24, 1.05)</td>
<td>0.078</td>
<td>90</td>
<td>0.47 (0.27, 0.82)</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Hertog 1997</td>
<td>UK (Caerphilly Study)</td>
<td>1,900 (M)</td>
<td>45 to 59</td>
<td>14</td>
<td>MI &amp; IHD death</td>
<td>FFQ/Mostly local analysis</td>
<td>Relative risk by quartiles</td>
<td>Flavone + flavonol: ≤19 vs &gt;34</td>
<td>186</td>
<td>1.1 (0.6, 1.6)</td>
<td>0.996</td>
<td>131</td>
<td>1.6 (0.9, 2.9)</td>
<td>0.119</td>
<td></td>
</tr>
<tr>
<td>Rimm 1996</td>
<td>USA (Health Professional Follow-up)</td>
<td>34,789 (M)</td>
<td>40 to 75</td>
<td>6</td>
<td>MI &amp; Revascularization or CHD death</td>
<td>FFQ/Mostly local analysis</td>
<td>Relative risk by quintiles</td>
<td>Flavone + flavonol: ≤7.1 vs ≤40.0</td>
<td>373</td>
<td>0.94 (0.68, 1.31)</td>
<td>—</td>
<td>140</td>
<td>0.77 (0.45, 1.35)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Continued: Summary of prospective cohort studies investigating the association of flavonoid intake with ischemic heart disease

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Baseline age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Analysis</th>
<th>Intake (low vs high) mg/day</th>
<th>Outcome</th>
<th>Intake estimate (95% CI)</th>
<th>P for trend</th>
<th>Intake estimate (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knekt 1996&lt;sup&gt;x&lt;/sup&gt;</td>
<td>Finland (Finnish Mobile Clinic)</td>
<td>M: 2,748 F: 2,385</td>
<td>30 to 69</td>
<td>26</td>
<td>410–414</td>
<td>Diet history/Mostly Hertog et al.&lt;sup&gt;38,39&lt;/sup&gt;</td>
<td>Relative risk by quartiles</td>
<td>Males: Flavone + flavonol: &lt;2.1 vs &gt;4.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>324</td>
<td>0.67 (0.44, 1.00)</td>
</tr>
<tr>
<td>Hertog 1993&lt;sup&gt;y&lt;/sup&gt;</td>
<td>Netherlands (Zutphen)</td>
<td>805 (M)</td>
<td>65 to 84</td>
<td>5</td>
<td>Incidence: MI Mortality: ICD</td>
<td>Diet history/Mortality: ICD Hertog et al.&lt;sup&gt;38,39&lt;/sup&gt;</td>
<td>Relative risk by tertiles</td>
<td>Flavone + flavonol: &lt;2.4 vs &gt;5.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>149</td>
<td>0.73 (0.41, 1.32)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Diet data capture method/Major flavonoid database used. <sup>b</sup>Subclass composition and calculations of flavonoid intake may differ between studies; the term ‘Total’ is used to denote the sum of 5 or more flavonoid subclasses. <sup>c</sup>Incidence is defined as the first fatal or non-fatal event unless otherwise indicated. <sup>d</sup>The model with the most covariate adjustments in each manuscript is reported; covariates differ between manuscripts. *Includes non-fatal events only. **Flavonoid intake estimated as found in food (i.e., mostly glycosides). †Represents the association for a proportional difference in flavonoid intake (see corresponding analysis description). Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CHD, coronary heart disease; EPIC, European Prospective Investigation into Cancer and Nutrition; F, female; FFQ, food frequency questionnaire; ICD, International Classification of Disease; BHD, ischemic heart disease; M, male; MI, myocardial infarction; NHS, Nurses’ Health Study; ODC, other derived compounds (e.g., theaflavins and/or thearubigins); PA, proanthocyanidin; PE, Phenol-Explorer; PHS, Public Health Study; REGARDS, The REasons for Geographic and Racial Differences in Stroke; USA, United States of America; USDA, United States Department of Agriculture; UK, United Kingdom; WHS, Women’s Health Study; WHO, World Health Organisation; μ, mean; κ, median.
c. Cerebrovascular disease

Disease overview. Cerebrovascular disease is the second leading cause of death and the leading cause of adult long-term disability. Cerebrovascular disease is the collective term for a broad range of conditions that adversely affect cerebral blood supply including stroke, transient ischemic attacks, other intracranial vascular disorders as well as late effects of stroke or its sequelae. Stroke is the primary sub-type of cerebrovascular disease and is characterised by a sudden onset of neurological dysfunction. It is typically caused by an occlusion in the cerebral vasculature resulting in an ischemic stroke and, less often, vessel rupture producing a haemorrhagic stroke. In high income countries, such as within North America, death from ischemic stroke accounts for as high as 67.9% of stroke mortality. Likewise, the prevalence of ischemic stroke dominates hemorrhagic strokes in Australasia and Western and Central Europe, reaching as high as 74.3% of stroke cases in North America. Vessel occlusion, occurring in ischemic stroke, is mostly a consequence of atherosclerosis either locally, producing luminal narrowing precipitating obstruction, or from atherosclerotic thrombus or emboli, which form elsewhere in the body, dislodge and travel to the cerebrovasculature, where they cause significant vascular ischemia. As flavonoids have shown anti-atherogenic and anti-thrombotic properties, they are thought to play a role in mitigating the pathogenesis and progression of stroke from atherosclerotic origins. While most literature on flavonoid intake and cerebrovascular events has included the broad range of cerebrovascular disease in their assessment of outcome cases, as highlighted above, these are principally ASCVD related.

Cohort characteristics. At least 18 prospective cohorts (in 21 publications) have reported on flavonoid intake and risk of cerebrovascular disease incidence and/or mortality (Table 3). Some of these cohorts are reported across multiple publications, such as the Zutphen Elderly study and Iowa Women’s Health Study, reflecting either the examination of certain flavonoids after different lengths of follow-up, or the examination of different flavonoids, in different
manuscripts. Across all studies, incidence was defined as the first non-fatal or fatal event. One study restricted analyses to first time events resulting in hospitalisation.62 Outcome events were mostly defined as all cerebrovascular disease using ICD-9 codes 430–438 or their equivalent, to identify cases. Though, some studies reported exclusively on ischemic stroke.55,62,129,130 Others reported across multiple types of cerebrovascular disease, for which we present findings for ischemic stroke in Table 3, as flavonoids appear most relevant to ASCVD.35,47,119,131 Overall, cohorts were conducted in Europe (n = 10), the United States of America (n = 6), and Asia (n = 2), and they vary in size (from 552 to 98,469 participants) and follow-up periods (from 4.9 to 28 years). Flavonoid intake was mostly estimated from FFQs (n = 12 publications) and diet histories (n = 6 publications) using the USDA database (n = 6 publications), Phenol-Explorer (n = 2 publications), tables by Hertog et al. (n = 3 publications) and Arts et al. (n = 2 publications), among a variety of other sources. Across all studies, only one indicated that flavonoid intake was estimated as glycosides (and other naturally occurring compounds);60 all other studies appear to report intake as AE (including free form and potentially esterified flavan-3-ols).

**Total flavonoids.** Total flavonoid intake (estimated as ≥5 subclasses) has been examined in 6 cohorts for an association with cerebrovascular disease incidence or mortality. In most cohorts there was a trend towards a lower cerebrovascular disease risk with higher consumption. However, none reached statistical significance in the highest exposure category. In 2016, Tang et al. published a meta-analysis which pooled 11 cohorts (n = 356,627; n = 5,154 cases), reporting across incidence and mortality measures of cerebrovascular disease (n = 8 cohorts) and ischemic stroke (n = 3 cohorts), with any flavonoid exposure; from total intake (n = 3 cohorts), to specific subclasses or their combinations (n = 8 cohorts).132 In their main analysis, the authors report a significantly lower risk of cerebrovascular disease for persons with higher flavonoid intake (multivariable adjusted risk estimate for highest vs lowest intake category:
In their sub-analysis of ischemic stroke, the association was not as clear (n = 3 cohorts, n = 98,069; multivariable adjusted risk estimate for highest vs lowest flavonoid intake category: 0.93, 95% CI: 0.80, 1.07; $I^2 = 0\%$). However, these results are limited by the number of cohorts pooled. Consequently, while the body of evidence suggests an association between flavonoid intake and cerebrovascular disease risk, further research re-orientated towards disease sub-types, such as ischemic stroke, may help facilitate a better granularity of evidence.

Flavonoid subclasses. Individual flavonoid subclasses have been examined for associations with cerebrovascular disease incidence (n = 15 cohorts across 16 publications) and mortality (n = 4 cohorts across 6 publications). Across cohorts, a trend towards a lower risk of cerebrovascular disease emerges, reaching significance in at least one or more cohorts, for all subclasses, except flavones (Table 3). By 2010, Hollman et al. had pooled 6 cohorts (n = 111,067, n = 2,155 cases), reporting intake combinations of flavonols, flavones and flavanones, and outcomes events of cerebrovascular disease and ischemic stroke, across incidence and mortality measures. They found, overall, a lower risk of cerebrovascular disease in those participants consuming the highest compared to the lowest estimated intakes (multivariable adjusted risk estimate: 0.80, 96% CI: 0.65, 0.98). However, heterogeneity ($I^2 = 54\%$) and publication bias (Eggers test = 0.01) was present. In 2014, Wang et al. conducted an updated systematic review and meta-analysis, similar that of Hollman et al., including two further cohorts, which substantially increased the sample size and number of cases (n = 280,174, n = 5,228 cases). Again a significantly lower risk was observed (multivariable adjusted risk estimate for highest vs lowest intake category: 0.86, 96% CI: 0.75, 0.99) though, again, publication bias was indicated (Eggers test = 0.005). Wang et al. further conducted sub-analyses, finding a trend towards a lower risk of ischemic stroke events (n = 4 cohorts; multivariable adjusted risk estimate for highest vs lowest intake category: 0.86, 95% CI: 0.71,
1.04; $I_2 = 48\%$), and unsurprisingly, given disease aetiology, this trend was less apparent for haemorrhagic stroke events ($n = 3$ cohorts; multivariable adjusted risk estimate: 0.90, 95% CI: 0.61, 1.32; $I_2 = 50\%$).\textsuperscript{134} In 2019, Kimble et al. pooled ten cohorts reporting incidence or mortality outcomes of cerebrovascular disease or specific stroke sub-types, finding no clear association with higher intake of anthocyanins or berries which were used as a surrogate indicator of anthocyanin intake (multivariable adjusted risk estimate for highest vs lowest flavonoid intake category: 1.00, 95% CI: 0.93, 1.07; $I_2 = 15.1\%$).\textsuperscript{109} When analysed by disease sub-type, there was a trend towards a lower risk of ischemic stroke with higher anthocyanin/berry consumption ($n = 4$ cohorts; multivariable adjusted risk estimate for highest vs lowest flavonoid intake category: 0.90, 95% CI: 0.79, 1.03; $I_2 = 0\%$).\textsuperscript{109} However, no such association was apparent between anthocyanin intake and haemorrhagic stroke ($n = 2$ cohorts; multivariable adjusted risk estimate for highest vs lowest flavonoid intake category: 1.01, 95% CI: 0.75, 1.35; $I_2 = 0\%$).\textsuperscript{109} A meta-analysis has also been performed on cohort studies reporting on flavan-3-ol intake and incident cerebrovascular disease; while no association was found, the analysis is limited by the number of included cohorts ($n = 2$ cohorts; multivariable adjusted risk estimate for highest vs lowest flavonoid intake category: 0.95; 95% CI: 0.80, 1.12; $I_2 = 0.0\%$).\textsuperscript{86} However, higher flavan-3-ol monomer intake was associated with a significantly lower risk of cerebrovascular disease ($n = 4$ cohorts; multivariable adjusted risk estimate for highest vs lowest flavonoid intake category: 0.82; 95% CI: 0.68, 0.99; $I_2 = 0.0\%$).\textsuperscript{86} Thus, at this time, additional research is needed to clearly delineate the role of all flavonoid subclasses on cerebrovascular disease risk and its distinct disease sub-types.

**Effect modification.** The beneficial association between flavonoid-rich diets and cerebrovascular disease might differ across population subgroups. Goetz et al. evaluated 20,024 participants in the REGARDS study and tested for effect modification of flavonoids on incidence of ischemic stroke by race (non-Hispanic white and black Americans) and sex.\textsuperscript{129
After 6.5 years of follow-up (and 524 cases) no significant effect modification by race or sex was observed for any flavonoid exposure including all major subclasses and their total intake; though corroboration of these findings in further samples is needed.\textsuperscript{129} In the Nurses’ Health Study \((n = 69,622)\) effect modification by hypertension and aspirin use was tested \((n = 1,803\) cases) and no interactions were observed after 14 years of follow-up.\textsuperscript{131} In contrast, in the Health Professionals Follow-Up Study, after 24 years of following 43,880 participants, significant effect modification by age occurred, wherein higher flavanone intake was associated with a lower risk of incidence ischemic stroke in men aged \(>65\) years \((HR: 0.71, 95\% CI: 0.54, 0.92)\) compared with men \(\leq 65\) y \((HR: 1.0, 95\% CI: 0.66, 1.51; P_{interaction} = 0.04)\), presumably due to a higher rate of death among the more elderly.
### Table 3. Summary of prospective cohort studies investigating the association of flavonoid intake with cerebrovascular disease

<table>
<thead>
<tr>
<th>Author, year (cohort)</th>
<th>Country (population)</th>
<th>Base-line age (years)</th>
<th>Follow-up (years)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Analysis</th>
<th>Exposure&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Intake (low vs high) (mg/day)</th>
<th>Cerebrovascular disease incidence&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Cerebrovascular disease mortality</th>
<th>P for trend</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalgaard 2019&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Denmark (Danish Diet, Cancer &amp; Health study)</td>
<td>M: 3,552 (M &amp; F)</td>
<td>56</td>
<td>Hospital admission for ICD-10 J63</td>
<td>FFQ&lt;sup&gt;e&lt;/sup&gt;/ PE</td>
<td>Quintile one vs 1000 mg</td>
<td>Total: x&lt;sup&gt;1&lt;/sup&gt; = 174 vs 1000</td>
<td>2,920 0.91 (0.82, 1.01)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adriaouch 2018&lt;sup&gt;e&lt;/sup&gt;</td>
<td>France (Nutrinet-Sante)</td>
<td>M: 84,158 (M &amp; F)</td>
<td>44.1</td>
<td>Stroke 3 x 24 hr diet record/ PE&lt;sup&gt;***&lt;/sup&gt;</td>
<td>Hazard ratio by tertiles</td>
<td>Anthocyanin: x&lt;sup&gt;2&lt;/sup&gt; = 27.5 vs x&lt;sup&gt;2&lt;/sup&gt; = 55.0</td>
<td>293 0.61 (0.43, 0.85)</td>
<td>0.007</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cassidy 2016&lt;sup&gt;f&lt;/sup&gt;</td>
<td>USA (Health Professionals Follow-Up)</td>
<td>M: 43,880 (M)</td>
<td>32</td>
<td>National Survey of Stroke criteria (M)</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyannidins: x&lt;sup&gt;2&lt;/sup&gt; = 1.9 vs x&lt;sup&gt;2&lt;/sup&gt; = 26.3</td>
<td>200 0.93 (0.75, 1.15)</td>
<td>0.51</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dower 2016&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Netherlands (Zutphen)</td>
<td>M: 774 (M)</td>
<td>65</td>
<td>Diet history/ Ains et al&lt;sup&gt;144&lt;/sup&gt;</td>
<td>Hazard ratio by tertiles</td>
<td>Catechin: x&lt;sup&gt;2&lt;/sup&gt; = 9.4 vs 19.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.73 (0.39, 1.38)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Goetz 2016&lt;sup&gt;h&lt;/sup&gt;</td>
<td>USA (REGARDS)</td>
<td>M: 20,024 (M &amp; F)</td>
<td>≥45</td>
<td>Medical records &amp; National Death</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanin: ≤4.8 vs 18.5</td>
<td>524 0.94 (0.71, 1.25)</td>
<td>0.67</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vogtitz-oglou 2015&lt;sup&gt;i&lt;/sup&gt;</td>
<td>UK (EPIC - Norfolk cohort)</td>
<td>M: 11,232 F: 13,633</td>
<td>11.1</td>
<td>7-day diary/ Flavonol Flavonol Food Database</td>
<td>Hazard ratio by quintiles</td>
<td>Males: Total flavan-3-ol: x&lt;sup&gt;2&lt;/sup&gt; = 198 vs x&lt;sup&gt;2&lt;/sup&gt; = 2008</td>
<td>955 1.08 (0.82, 1.43)</td>
<td>0.727</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tabei 2014&lt;sup&gt;j&lt;/sup&gt;</td>
<td>Singapore (Singapore Chinese Health Study)</td>
<td>M: 65,257 (M &amp; F)</td>
<td>45</td>
<td>ICD-9</td>
<td>Hazard ratio by quartile</td>
<td>Total flavan-3-ol: x&lt;sup&gt;2&lt;/sup&gt; = 179 vs x&lt;sup&gt;2&lt;/sup&gt; = 1828</td>
<td>605 0.89 (0.68, 1.19)</td>
<td>0.384</td>
<td>1,298 0.97 (0.81, 1.16)</td>
<td>0.41</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Author, year</td>
<td>Country (cohort)</td>
<td>n (population)</td>
<td>Baseline age (yrs)</td>
<td>Follow-up (yrs)</td>
<td>Outcome assessment</td>
<td>Flavonoid intake estimate*</td>
<td>Analysis</td>
<td>Exposure</td>
<td>Intake (low vs high) mg/day</td>
<td>Cerebrovascular disease incidence†</td>
<td>Cerebrovascular disease mortality</td>
<td></td>
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<td>---------------------------------</td>
<td>--------------------------------</td>
<td></td>
</tr>
<tr>
<td>Cassidy 2012</td>
<td>USA (NHS)</td>
<td>69,622</td>
<td>30</td>
<td>14</td>
<td>National Survey of Stroke</td>
<td>FFQ USDA</td>
<td>Relative risk by quartiles</td>
<td>Anthocyanidin: &lt;5.4 vs &gt;20.2</td>
<td>943</td>
<td>0.89 (0.72, 1.11)</td>
<td>1.01 (0.78, 1.28)</td>
<td>0.64 (0.34, 1.24)</td>
</tr>
<tr>
<td>Mc-Cullough 2012</td>
<td>USA (Cancer Prevention Study II)</td>
<td>98,469</td>
<td>−69</td>
<td>7</td>
<td>ICD-9 430-438 &amp; ICD-10 160-169</td>
<td>FFQ USDA</td>
<td>Hazard ratio by quartiles</td>
<td>Anthocyanidin: &lt;5.5 vs ≥16.7</td>
<td>573</td>
<td>0.95 (0.75, 1.20)</td>
<td>0.90 (0.71, 1.14)</td>
<td>0.93 (0.74, 1.16)</td>
</tr>
<tr>
<td>Mursu 2008</td>
<td>Finland (Kuopio IHD study)</td>
<td>1,950</td>
<td>42</td>
<td>15.2</td>
<td>ICD-9 433-434 &amp; ICD-10 163</td>
<td>4-day diary/ Local database USDA</td>
<td>Relative risk by quartiles</td>
<td>Anthocyanidin: &lt;7.6 vs ≥253.6</td>
<td>0.88 (0.70, 1.10)</td>
<td>1.00 (0.78, 1.28)</td>
<td>0.83 (0.66, 1.04)</td>
<td></td>
</tr>
<tr>
<td>Kobudo 2007</td>
<td>Japan (Japanese PHS Cohort II)</td>
<td>40,462</td>
<td>48</td>
<td>12</td>
<td>ICD-10 160-161, I63 &amp; I69</td>
<td>FFQ/Local database</td>
<td>Hazard ratio by quartiles</td>
<td>Males: Anthocyanidin: &lt;9 vs ≥33.6</td>
<td>308</td>
<td>0.83 (0.66, 1.04)</td>
<td>0.83 (0.66, 1.04)</td>
<td>0.83 (0.66, 1.04)</td>
</tr>
<tr>
<td>Mink 2007</td>
<td>USA (Iowa WHS)</td>
<td>34,489</td>
<td>55</td>
<td>16</td>
<td>ICD-9 430-438</td>
<td>FFQ USDA</td>
<td>Relative risk by quartiles (except anthocyanidin by 2 groups)</td>
<td>Anthocyanidin: &lt;8 vs ≥0.01</td>
<td>160</td>
<td>0.95 (0.70, 1.31)</td>
<td>0.95 (0.70, 1.31)</td>
<td>0.95 (0.70, 1.31)</td>
</tr>
<tr>
<td>Harnainen 2005</td>
<td>Finland (no name)</td>
<td>755</td>
<td>65</td>
<td>10</td>
<td>ICD-9 431, 432, 433, 434, 436</td>
<td>Diet history/ Nutrica</td>
<td>Relative risk by tertiles</td>
<td>Flavone: &lt;0.9 vs ≥1.4</td>
<td>469</td>
<td>1.01 (0.83, 1.24)</td>
<td>1.01 (0.83, 1.24)</td>
<td>1.01 (0.83, 1.24)</td>
</tr>
<tr>
<td>van der Schouw 2005</td>
<td>Netherlands (Prospect EPIC)</td>
<td>16,165</td>
<td>49</td>
<td>6.25</td>
<td>ICD-9 430-438</td>
<td>FFQ self-developed database</td>
<td>Hazard ratio by quartiles</td>
<td>Anthocyanidin: &lt;2 vs ≥5</td>
<td>147</td>
<td>1.05 (0.64, 1.70)</td>
<td>1.05 (0.64, 1.70)</td>
<td>1.05 (0.64, 1.70)</td>
</tr>
</tbody>
</table>
### Table 3. Continued: Summary of prospective cohort studies investigating the association of flavonoid intake with cerebrovascular disease

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Base-line age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Analysis</th>
<th>Exposure&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Intake (low vs high) mg/day</th>
<th>Cerebrovascular disease incidence&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P for trend</th>
<th>Cerebrovascular disease mortality</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesso 2003&lt;sup&gt;14&lt;/sup&gt;</td>
<td>USA (WHS)</td>
<td>38,445 (F)</td>
<td>&gt;45</td>
<td>6.9</td>
<td>Stroke</td>
<td>FFQ/ Mostly Hertog et al.&lt;sup&gt;38,39&lt;/sup&gt;</td>
<td>Relative risk by quintiles</td>
<td>Flavone + flavonol: &lt;i&gt;x̄&lt;/i&gt;=8.8 vs &lt;i&gt;x̄&lt;/i&gt;=47.4</td>
<td>70.0 (0.46, 1.07)</td>
<td>0.43</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vokó 2003&lt;sup&gt;16&lt;/sup&gt;</td>
<td>Netherlands (Rotterdam study)</td>
<td>5,197 (M &amp; F)</td>
<td>67.6</td>
<td>6.4</td>
<td>Review by neurologist for ischemic stroke</td>
<td>FFQ/ Composite of older sources</td>
<td>Relative risk by tertiles</td>
<td>Flavonoid (undefined):</td>
<td>277.0 (0.66, 2.12)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Knekt 2002&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Finland (Fiinland)</td>
<td>9,131 (M &amp; F)</td>
<td>39.3</td>
<td>28</td>
<td>ICD-8 for thrombotic stroke</td>
<td>Diet history/ mostly local analysis</td>
<td>Relative risk by quartiles</td>
<td>Flavone + flavonol + flavanone:</td>
<td>423.0 (0.54, 0.98)</td>
<td>0.004</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Arts 2001&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Netherlands (Zutphen)</td>
<td>806 (M)</td>
<td>65 to 84</td>
<td>10</td>
<td>ICD-9</td>
<td>Diet history/ Arts et al.&lt;sup&gt;43,44&lt;/sup&gt;</td>
<td>Risk ratio by tertiles</td>
<td>Catechin: &lt;i&gt;x̄&lt;/i&gt;=49.0 vs ≥85.9</td>
<td>488.0 (0.51, 1.68)</td>
<td>0.749</td>
<td>47</td>
<td>0.81 (0.36, 1.83)</td>
<td>0.606</td>
</tr>
<tr>
<td>Hirvonen 2000&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Finland (ATBC Study)</td>
<td>26,593 (M smokers)</td>
<td>50 to 69</td>
<td>6.1</td>
<td>ICD-8 for cerebral infarction</td>
<td>Diet history/ Mostly Hertog et al.&lt;sup&gt;38,39&lt;/sup&gt;</td>
<td>Relative risk by quartiles</td>
<td>Flavone + flavonol: &lt;i&gt;x̄&lt;/i&gt;=4.2 vs ≥16.4</td>
<td>736.0 (0.80, 1.21)</td>
<td>0.81</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Yochum 1999&lt;sup&gt;20&lt;/sup&gt;</td>
<td>USA (Iowa WHS)</td>
<td>34,492 (post-menopause women)</td>
<td>61 to 10</td>
<td>10</td>
<td>ICD-9</td>
<td>Diet history/ Mostly Hertog et al.&lt;sup&gt;38,39&lt;/sup&gt;</td>
<td>Relative risk by quartiles</td>
<td>Flavone + flavonol: &lt;i&gt;x̄&lt;/i&gt;=5.7 vs ≥18.7</td>
<td>438.0 (0.70, 2.00)</td>
<td>0.83</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Keli 1996&lt;sup&gt;,21&lt;/sup&gt;</td>
<td>Netherlands (Zutphen)</td>
<td>552 (M)</td>
<td>50 to 69</td>
<td>15</td>
<td>ICD-9</td>
<td>Diet history/ Netherlands food table</td>
<td>Relative risk by tertile</td>
<td>Flavone + flavonol: &lt;i&gt;x̄&lt;/i&gt;=18.3 vs ≥28.6</td>
<td>42.0 (0.11, 0.70)</td>
<td>0.004</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup>Diet data capture method/Major flavonoid database used. <sup>b</sup>Subclass composition and calculations of flavonoid intake may differ between studies; the term “Total” is used to denote the sum of 5 or more flavonoid subclasses. <sup>c</sup>Incidence is defined as the first fatal or non-fatal event unless otherwise indicated. <sup>d</sup>The model with the most covariate adjustments in each manuscript is reported; covariates differ between manuscripts. **Flavonoid intake estimated as found in food (i.e., mostly glycosides). Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; EPIC, European Prospective Investigation into Cancer and Nutrition; F, female; FFQ, food frequency questionnaire; ICD, International Classification of Disease; IHD, ischemic heart disease; M, male; NHS, Nurses’ Health Study; ODC, other derived compounds (e.g, theaflavins and/or thearubigins); PA, proanthocyanins; PE, Phenol-Explorer; PHS, Public Health Study; REGARDS, The REasons for Geographic and Racial Differences in Stroke; USA, United States of America; USDA, United States Department of Agriculture; UK, United Kingdom; WHS, Women’s Health Study; <i>x̄</i>, mean; <i>x̄</i>, median.
Peripheral artery disease

Disease overview. It is estimated that PAD is the most prevalent CVD globally, though PAD mortality accounted for a mere ~0.3% of total CVD-related death in 2015. In general PAD refers to acute or chronic obstruction of the arteries supplying the lower/upper extremities, excluding those supplying the heart or brain. The most common cause of PAD is atherosclerosis, which accounts for >90% of cases. Individuals with PAD have a higher risk of coronary and cerebral atherosclerosis, and are several times more likely to experience myocardial infarction or ischemic stroke than individuals without PAD. The clinical spectrum of PAD is wide, including people who are asymptomatic (~50% of those affected); to those experiencing intermittent claudication (~10 to 30% of PAD patients), wherein pain in the calves occurs on exercise and is relieved by rest. The most severe progression of PAD is critical limb ischemia, which develops in ~1 to 2% of PAD patients, leading to ulceration and gangrene requiring amputation in ~30% of patients, and ultimately causing death in 25% of those affected within one year. Evidence suggests flavonoids attenuate pathophysiological processes involved in PAD, and consequently, we sought to report on cohorts describing the association of flavonoids with risk of PAD.

Cohort characteristics and findings. To our knowledge, only two prospective cohorts (in three publications), have investigated the association of flavonoid intake with PAD risk. As early as 2004, Hirvonen et al. evaluated the risk of intermittent claudication, after 4.1 years of follow-up, in 25,041 male smokers, recruited to the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. The authors observed a significant inverse association in their age-adjusted, and lifestyle-adjusted models, yet, upon further adjustment for intake of vitamins C, E and carotenoids, significance was attenuated, yet a trend towards a lower risk of intermittent claudication, with higher intakes of flavonols plus flavones remained (Table 4). More recently, Bondonno et al. studied the risk of hospitalisation for PAD in the Danish Diet, Cancer & Health study of >50,000 people, over >20 of follow-up (in press). In their earlier manuscript,
PAD is reported as a secondary outcome, and a comparison between total flavonoid intake of 175 mg/day (quintile one) and 1000 mg/day is made, showing a statistical significantly lower associated risk of PAD (multivariable adjusted HR: 0.68, 95% CI: 0.60, 0.78).\textsuperscript{62} In their latter manuscript (in press), the authors went on to study PAD in its entirety. In the same cohort, after adjustment for lifestyle and dietary factors, a lower risk of PAD was observed, across all subclasses, reaching significance for intake of anthocyanin, catechins, proanthocyanidins, flavonols and total flavonoids (Table 4). The non-linear association between total flavonoid intake and PAD hospitalizations plateaued at ~750 to 1000 mg/day after which no added benefit was observed. Moreover, across secondary endpoints, compared to a total flavonoid intake of 174 mg/day, for intakes of 1000 mg/day and 500 mg/day respectively, a lower risk of revascularizations/endovascular surgery [HR: 0.57 (95% CI 0.46, 0.69)], and amputations [HR: 0.56 (95% CI 0.43, 0.74)] was observed. Furthermore, on both a relative and an absolute scale, the greatest benefits observed for higher flavonoid intakes were in current or former smokers. Overall, evidence is sparse yet promising, that higher intake of flavonoid rich foods lowers risk of PAD.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country (cohort)</th>
<th>n (population)</th>
<th>Baseline age (yrs)</th>
<th>Follow-up (yrs)</th>
<th>Outcome assessment</th>
<th>Flavonoid intake estimate</th>
<th>Analysis</th>
<th>Exposure</th>
<th>Intake (low vs high) mg/day</th>
<th>Fully adjusted estimate (95% CI) [high vs low]</th>
<th>P for trend</th>
<th>n</th>
<th>Fully adjusted estimate (95% CI) [high vs low]</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bondonno 2020</td>
<td>Denmark (Danish Diet, Cancer &amp; Health study)</td>
<td>56,048</td>
<td>56</td>
<td>23</td>
<td>Hospital admission for ICD-10 I70- I74</td>
<td>FFQ/PE</td>
<td>Hazard ratio by quintiles</td>
<td>Anthocyanidin: &lt;10 vs ≥53</td>
<td>2,131</td>
<td>0.86 (0.76, 0.98)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dalgaard 2019</td>
<td>Denmark (Danish Diet, Cancer &amp; Health study)</td>
<td>53,552</td>
<td>56</td>
<td>23</td>
<td>Hospital admission for ICD-10 I70- I74</td>
<td>FFQ/PE</td>
<td>Quintile one vs 1000 mg</td>
<td>Total: &lt;251 vs ≥509</td>
<td>1,867</td>
<td>0.68 (0.60, 0.78)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hirvonen 2004</td>
<td>Finland (ATBC Study)</td>
<td>25,041</td>
<td>50</td>
<td>4.1</td>
<td>1C using Rose questionnaire</td>
<td>Diet history/ Mostly Hertog et al.18</td>
<td>Relative risk by quintiles</td>
<td>Flavonol + flavonol: —</td>
<td>2,412</td>
<td>0.93 (0.81, 1.08)</td>
<td>0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Comments [BP2]: Reviewers to note: This article has been accepted for publication and is in press. The reference will be added and in press removed before publication of this review. (note: once added, this make 44 publications. Text is correct as: "27 prospective cohorts in 44 publications").
6. Discussion: current evidence—future epidemiological frontiers

In the absence of long-term RCTs, the prospective cohort studies conducted to date offer critical information on the typical foods and beverages consumed by populations, their relative flavonoid contents, and the long-term association with CVD incidence and mortality. Over the past three decades, at least 27 prospective cohorts (in 44 publications) have evaluated the relationship between estimated flavonoid intake and CVD risk. While inconsistencies in findings are observed between studies, meta-analyses of these cohorts tend to suggest a common significant association, wherein, habitual, long-term intakes of flavonoid-rich diets may be associated with a lower risk of IHD, cerebrovascular disease, and total CVD. Less evidence is available for PAD. Of the flavonoid subclasses investigated, epidemiological evidence more often supports diets rich in anthocyanins, flavan-3-ols, and flavonols in lowering the risk of chronic CVD. At the same time, several mechanisms have been investigated by which flavonoids might modulate the causal pathway of major CVD. To this end, short-term RCTs provide evidence that flavonoids lower blood pressure, improve endothelial function, improve arterial stiffness, reduce dyslipidaemia, and mediate inflammatory responses. As these mechanisms are collectively implicated in atherosclerosis, they are thought, among other possible mechanisms, to conjointly influence the trajectory of chronic CVD, particularly, ASCVD. Seemingly, converging evidence of the effects of flavonoids on intermediate endpoints and their association with hard clinical outcomes is suggestive that flavonoid-rich foods, may be protective against CVD. If confirmed, food policy strategies incorporating evidence of the benefits of flavonoid-rich diets may have important implications for health care services and public health outcomes.

While meta-analyses tend to suggest a significant inverse association between flavonoid intake and CVD risk, some inconsistencies in findings between the cohort studies seem apparent. There are multiple potential reasons for these discrepancies, including differences in demographic
characteristics or study settings, differences in the actual absolute intake between populations, the length of follow-up, the varying definition of outcomes, accuracy of covariate assessment, heterogeneous covariate adjustments, the sample size (and number of cases) and consequently, adequate statistical power for meaningful conclusions. Moreover, U-shaped associations have been seen, wherein participants with moderate intakes have a significantly lower risk of CVD, but for those in the highest intake category, this association is not significant. The reason for this finding is not entirely clear, but fewer individuals with these high intakes means less certainty (wider confidence intervals), and findings must be interpreted with caution. In addition, the studies used different flavonoid databases at varying stages of their 'development' which may have also contributed to varying outcomes; to this end, the known limitations of using databases to calculate dietary intake also apply. Further, dietary assessment tools also contribute to measurement error in the exposure. Many of the studies summarised in this review used FFQs, and many of these FFQs have not been validated specifically for flavonoids. Though, even if they were, such self-reported dietary data would still contain measurement error and this has long been acknowledged. Such uncertainty in exposure data translates into uncertainty of outcomes and biases associations towards the null raising the possibility of false-negatives. Thus, null findings may not necessarily predicate no association. Indeed, in meta-analyses of prospective cohorts, although often accompanied by statistically significant heterogeneity (likely from some of the aforementioned causes), an overall link between flavonoid-rich diets and IHD, cerebrovascular disease and total-CVD emerges. As the temporal relationship between the exposures and outcomes reveals that reverse causality is unlikely attributable, this supports the presence of a beneficial association in favour of flavonoid intake. However, whether the associations represent a benefit of the isolated bioactives per se, or a signal of the bioactives working in concert with the co-occurring nutrient matrix within flavonoid-bearing foods, are issues of consideration. Moreover, whether beneficial associations between total flavonoid intake and CVD is driven by flavan-3-ols, the
principal subclass consumed in most populations, or whether it represents the breakdown of several subclasses into common downstream bioactive metabolites, must be considered. Even the biological rationale for examining individual subclasses is somewhat tenuous as differences in the bioavailability and bioactivity of individual compounds within the same subclass is observed. Indeed, a central limitation of quantifying flavonoid intake based on aglycones is that the varying effects of different glycosides cannot be taken into consideration; this is best investigated in clinical trials and animal studies. Thus, while the calculation and correlation of aglycones with endpoints might be useful to obtain a broad overview of the relationships, there are several caveats with this approach. Thus, the simple interpretation of the data to date, and the one most relevant for dietary advice, is that consumption of flavonoid-rich foods or diets higher in flavonoids, appear nutritionally beneficial in the prevention of CVD.

Although, if persons with higher flavonoid intakes tend to have other positive dietary habits, one may question whether associations observed can be attributable to flavonoid-rich foods or whether these diets are simply a marker of higher diet quality overall. To address this issues, one approach has been to add potential dietary confounders as covariates in multivariable adjusted models, or to adjust for a “diet-quality” index. While the former approach is sensible when the dietary confounders are not also a source of flavonoids, risk estimates arising from models adjusting for flavonoid sources, such as fruits and vegetables, become difficult to interpret. Using a different approach, Bondonno et al. report evidence of an association between total flavonoid intake and all-cause mortality amongst participants in the highest fruit and vegetable intake tertile, suggesting that there may be a benefit to recommending a diet rich in flavonoids, above and beyond a diet rich in fruits and vegetables. This is reasonable as it is possible to have a diet low in fruits and vegetables but high in flavonoids and vice versa. Thus, the observed associations may not necessarily be a mere marker of higher overall diet quality, although, we are unable to rule out this hypothesis. Yet, to this end, we postulate that
higher intakes of flavonoid-rich foods are likely one factor of a healthy diet, which when coupled with other beneficial dietary habits, are likely to produce the greatest health benefits.

Comprehensive knowledge on the current state of the science is a prerequisite for hypothesis formulation and the design and conduct of further research. This review traces the development and advances of flavonoid epidemiology providing a broad overview and update on the current state of the science. To the best of our knowledge we include all major studies (written in English) and our general conclusions are in alignment with other reviews in the field, however, we limit our scope to intake of total flavonoids and major subclasses and selected CVD outcomes. To this end, a growing body of studies have also addressed individual flavonoid compounds and their relationship to CVD. These compounds may warrant further research, where intake is sufficiently variable and at potentially biologically relevant quantities in the populations being studied. To this end, a growing body of cohort studies have also recently addressed flavonoid intake and other CVDs or related risk factors, including, yet not limited to, risk of hypertension, type 2 diabetes, and overweight and obesity, which in general, show a protective association across such non-communicable conditions related to CVD.

In considering the design of further research, careful selection of lifestyle confounders is an issue of importance. This is because dietary intake may relate in a complicated manner to other social and behavioural determinants of disease. To date, most investigators have adjusted for age, sex (in mixed cohorts), body mass index, physical activity, and smoking history while, sometimes, indices of cardio-metabolic health, such as blood pressure, cholesterol and/or diabetes were incorporated. Not all studies adjusted for indices of socioeconomic status (SES), be it via education, income or profession. As flavonoid intake appears to be associated with SES, which in turn associated with CVD, there is reason to consider adjustment for SES in future studies. Though of course, confounders within cohorts will depend on the specific
population recruited. Most authors adjusted for total energy intake, via either a standard multivariable model or another method (e.g. nutrient residual). However some authors presented models without energy adjustment, as it is thought that absolute consumption (adjusted for body size), may be more relevant than energy-adjusted values. Although these studies tended to additionally conduct sensitivity analysis with energy included. Adjusting for energy intake made no difference, however, the interpretation of the result changes. Considerable heterogeneity is found in the current literature with regards to the adjustment of other dietary factor, with many authors not considering dietary cofounders at all. Thus, residual confounding in this regard likely to vary between studies.

Several knowledge gaps exist in the current evidence base; such gaps may need to be addressed if flavonoid epidemiology research is to inform dietary guidelines. There are only a few studies available on flavonoid intake and ischemic stroke as well as PAD and, as such, there is a need for further study in these areas. Moreover, although included under the umbrella of total CVD, flavonoid intake does not appear to be associated with atrial fibrillation, or haemorrhagic stroke; investigations targeting total ASCVD may be more appropriate than all-cause CVD, as flavonoids appear most relevant to CVDs with atherosclerotic origins. Including atrial fibrillation, which has a relatively high prevalence, within total CVD outcomes plausibly dilutes the association with flavonoid intake. Findings from the Danish Diet Cancer and Health Study cohort also suggest that a high flavonoid intake may be most beneficial to populations at risk of CVD, namely smokers and high alcohol consumers. Although similar effect modification by smoking or alcohol intake was not observed in the Nurses’ Health Study II or the REGARDS cohort; these analyses were specifically conducted in relation to IHD in contrast to total atherosclerotic CVD which was looked at in the Danish Diet Cancer and Health Study. A limitation of the Danish cohort is that the Danish population is more homogenous than many other countries, with most of the participants being Caucasian, limiting the generalizability of the findings to other ethnicities. Thus, further studies
are warranted to determine whether similar associations exist in other ethnic groups. As the inverse association between flavonoid intake and CVD may be stronger in subgroups of smokers and high alcohol consumers, the associations between flavonoid intake and CVD may differ in cohorts that have a higher proportion of these individuals. To this end, modification of the flavonoid-CVD association by other potential effect modifiers has been explored in a limited number of cohorts. No effect modification was found by sex, race, physical activity, education, aspirin use, type 2 diabetes mellitus, or body mass index, however, at least one study reported effect modification by age. As a result, it is also likely that the associations between flavonoid intake and CVD may appear stronger in cohorts that have a higher proportion of older individuals. Other differences which may also affect the association, such as length of follow-up or geographical region, are of interest. However, a recent meta-analysis found no difference in results, when subgroup analysis was performed on studies with these differing attributes (i.e., studies with less or greater than 10 years of follow-up and Mediterranean compared to non-Mediterranean countries) however, the number of studies included was small (n = 5). Corroboration of these findings in further samples is needed as is the examination of other potential effect modifiers.

In the context of advancing flavonoid research, the prospective cohort study appears to be the most feasible design realistically available for examining dietary exposures with long-term disease outcomes. Though of course, large-scale RCTs, where-ever viable, would immensely facilitate progress in this field. One long-awaited RCT, involving >20,000 participants, is the COocoa Supplement and Multivitamin Outcomes Study, which aims to examine the effect of flavan-3-ol intake on CVD outcomes, over 5 years of follow-up. Moving forward, the use of nutritional biomarkers or other innovative measures of flavonoid intake, in both RCTs and observational studies, provides a major future pathway for strengthening the investigation of flavonoids, by providing objective assessments of dietary intake or, in the case of biomarkers, further allowing for calibrated adjustment of subjective
intake values.\textsuperscript{169–171} There is a crucial need for quality biomarkers of intake as a means of overcoming well-known FFQ and food database limitations.\textsuperscript{172} As well as being subjective and restricted, the use of FFQs to estimate flavonoid exposure does not allow for differences in metabolism between individuals to be taken into consideration. This is crucial as metabolism affects the levels of circulating bioactive downstream metabolites and may be key in explaining why some individuals benefit from the consumption of flavonoid-rich foods whilst others do not. Furthermore, many cohort studies do not have FFQ data. Indeed, several recent meta-analyses of prospective cohorts using (poly)phenol biomarkers and assessing CVD outcomes, have shown a paucity of studies using this approach.\textsuperscript{173,174} With the ever-growing progress in identifying biomarkers of flavonoid exposure,\textsuperscript{68,170} this approach is likely to usher in a new era of research, with a combination of dietary data and biomarkers likely being the most favourable approach. One recent example is a case-control study by Murphy et al., whom used a newly developed method for quantifying 38 dietary (poly)phenols in plasma—seemingly the most comprehensive method for (poly)phenol biomarker assessment developed to date.\textsuperscript{175,176} Another important advantage of using biomarkers is their potential ability to capture exposure to foods and/or flavonoids, not included in food composition databases, such as food additives or dietary supplements.\textsuperscript{167} However, biomarkers are not without their own limitations and readers are directed to several key reviews for further discussion.\textsuperscript{68,170,171}

Future studies addressing several other limitations may also provide a more robust basis for evaluating the association between flavonoid intake and CVD. In prospective cohort studies lasting many years, repeated measures of dietary intake and other covariates would allow for cumulative exposures to be included, reducing regression dilution bias resulting from measurement error, and increasing confidence in the findings. One example can be seen in the Nurses’ Health Study II by Cassidy et al., where FFQs were collected every 4 years (as well as covariate data biennially) providing better estimates of average habitual flavonoid exposure (and covariate status) over the 18 year follow-up timeframe.\textsuperscript{56} 56 Other improvements include
increasing the reliability of the evidence base. Given the rising threat of the reproducibility crisis, cherry-picking and p-hacking, meta-analysis authors are encouraged to test for bias across all exposure-outcome assessments and are advised to include technical flavonoid experts on their team. While results of meta-analyses to date generally indicate that publication bias appears less prevalent in flavonoid research, both Hollman et al., and Wang et al., found evidence of publication bias, and thus caution is needed in interpreting the results on cerebrovascular disease. To facilitate improvements, epidemiologists are urged to adopt reporting guidelines for cohort studies, and flavonoid-specific research, as well as consider open access databases, and the pre-registration of analysis plans for cohort studies, thereby improving transparency and the reproducibility of research practices.

7. Conclusion

Nutrition plays a critical role in preserving cardiovascular health and lowering the risk of major CVD. In the early 1990’s, prospective studies began to emerge, reporting beneficial associations between a higher dietary flavonoid intake and a lower risk of CVD. Since this time a large volume of literature has accumulated. During the last ~25 years, the completeness of flavonoid-food composition databases have advanced substantially, although this brings with it inherent difficulty when cross-comparing (old and new) studies. This could explain some of the discrepancies between cohort studies, however difference in other cohort characteristics have likely also contributed. Differences in outcome definitions and the inclusion of total CVD cases, rather than those specifically of ASCVD, may have attenuated results, based on what is known on proposed flavonoid mechanism and atherosclerosis. Despite these limitations, the evidence from prospective cohort studies, when combined in meta-analyses, suggests, habitual, long-term moderate consumption of flavonoid-rich diets may be associated with lower risk of CVD. Specifically, the totality of evidence suggests a protective association between flavonoid intake and IHD, cerebrovascular disease and total CVD; disease outcomes which are
principally, though not exclusively, composed of cases of ASCVD. Limited available evidence also suggests a role of flavonoids in mitigating the risk of PAD and ischemic stroke although, further research is required. Of the subclasses investigated, evidence for a protective association appears strongest for diets rich in anthocyanin, flavan-3-ol, and flavonol intake. If confirmed, these findings have important clinical and public health implications as flavonoids are common in foods and CVD is of major public health concern. A major future pathway in studies of flavonoid intake is the use of nutritional biomarkers, in combination with traditional food intake approaches. Future epidemiological research re-orientated towards the investigation of ASCVD may enhance the evidence base as flavonoids are primarily hypothesised to lower the risk of CVD of atherosclerotic origins.
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