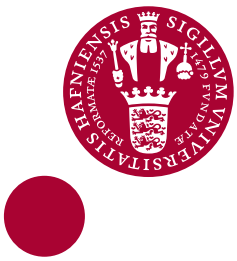

Associations between habitual flavonoid intake and hospital admissions for atherosclerotic cardiovascular disease

a prospective cohort study

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Associations between habitual flavonoid intake and hospital admissions for atherosclerotic cardiovascular disease: a prospective cohort study

Frederik Dalgaard, Nicola P Bondonno*, Kevin Murray, Catherine P Bondonno, Joshua R Lewis, Kevin D Croft, Cecilie Kyrø, Gunnar Gislason, Augustin Scalbert, Aedin Cassidy, Anne Tjønneland, Kim Overvad, Jonathan M Hodgson*

Summary

Background Flavonoids, compounds found in plant-based foods and beverages, might ameliorate vascular damage and atherosclerosis. Therefore, our aim was to assess the association between flavonoid intake and hospital admissions due to atherosclerotic cardiovascular disease.

Methods In this prospective cohort study, participants from the Danish Diet, Cancer, and Health Study were cross-linked with Danish nationwide registries. Eligible participants were aged 50–65 years, had no previous history of atherosclerotic cardiovascular disease, and had completed a food-frequency questionnaire at baseline. We examined associations between flavonoid intake (calculated from food-frequency questionnaires with use of the Phenol-Explorer database) and hospital admissions for atherosclerotic cardiovascular disease, ischaemic heart disease, ischaemic stroke, or peripheral arterial disease. We obtained hazard ratios (HRs) using restricted cubic splines based on Cox proportional hazards models.

Findings Of the participants recruited to the Danish Diet, Cancer, and Health study between 1993 and 1997, our study population was comprised of 53 552 participants, with a median follow-up of 21 years (IQR 15–22). During follow-up, 8773 participants were admitted to hospital for atherosclerotic cardiovascular disease. We observed non-linear associations between flavonoid intake and hospital admissions, plateauing at total flavonoid intakes of approximately 1000 mg per day. Compared with an intake of 175 mg per day, an intake of 1000 mg per day was associated with a 14% lower risk of atherosclerotic cardiovascular disease (HR 0·86, 95% CI 0·81–0·91). For disease subtypes, we observed a 9% lower risk of ischaemic heart disease (0·91, 0·85–0·98), a non-significant 9% lower risk of ischaemic stroke (0·91, 0·82–1·01), and a 32% lower risk of peripheral artery disease (0·68, 0·60–0·78). The overall associations were stronger in smokers than in non-smokers, as well as stronger in consumers of high (>20 g per day) quantities of alcohol than in those consuming low-to-moderate (≤20 g per day) quantities.

Interpretation Our results suggest that ensuring an adequate consumption of flavonoid-rich foods, particularly in subpopulations at risk of atherosclerosis such as smokers and consumers of high quantities of alcohol might mitigate some of the risk of atherosclerotic cardiovascular disease. More studies are needed to support and validate these data.

Funding Danish Cancer Society.

Introduction

The exploration of evidence-based approaches to prevent cardiovascular disease is crucial to combat the world's leading cause of death. Findings from the 2015 he Global Burden of Diseases, Injuries, and Risk Factors Study[1] indicate that, in Denmark, approximately 44% of deaths due to cardiovascular disease are associated with dietary risk factors. Globally, this proportion is closer to 56%, with the primary dietary risk factor associated with the development of cardiovascular disease being a low intake of fruits.[2]

One hypothesis to explain the cardioprotective effects of fruits and many vegetables is that these benefits are partly due to flavonoids, a group of bioactive compounds.[3] Flavonoids are also found in other plant-based foods and beverages such as tea, cocoa, and red wine and are categorised into subclasses on the basis of their chemical structure.[4] These structural variations yield differences in bioavailability and biological activity, making the individual assessment of the effects of these subclasses particularly informative.[5] However, flavonoid compounds from different subclasses are catabolised by the gut microbiome into smaller phenolic and aromatic acids that are common to several subclasses.[6] Therefore, assessing total flavonoid intake is also of value.

Small-scale, short-term intervention studies have explored the effects of flavonoids on validated measures of vascular health. These studies have shown that flavonoid-rich foods and specific flavonoid compounds can have a positive effect on surrogate markers of cardiovascular health such as blood pressure, vascular function, arterial stiffness, blood lipids, and inflammation.[7] Evidence

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*Contributed equally

Department of Cardiology, Herlev & Gentofte University Hospital, Copenhagen, Denmark (F Dalgaard MD, Prof G Gislason PhD); **School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia** (N P Bondonno PhD, C P Bondonno PhD, J R Lewis PhD, Prof J M Hodgson PhD); **School of Biomedical Sciences, University of Western Australia, Royal Perth Hospital, Perth, WA, Australia** (N P Bondonno, C P Bondonno, J R Lewis, Prof K D Croft PhD, Prof J M Hodgson); **School of Population and Global Health, University of Western Australia, Perth, WA, Australia** (K Murray PhD); **Danish Cancer Society Research Centre, Copenhagen, Denmark** (C Kyrø PhD, Prof A Tjønneland PhD); **National Institute of Public Health, University of Southern Denmark, Odense, Denmark** (Prof G Gislason); **Danish Heart Foundation, Copenhagen, Denmark** (Prof G Gislason); **International Agency for Research on Cancer, Lyon, France** (A Scalbert PhD); **Institute for Global Food Security, Queen's University Belfast, Belfast, UK** (Prof A Cassidy PhD); **Department of Public Health, Aarhus University, Aarhus, Denmark** (Prof K Overvad PhD); **and Faculty of Medicine, Aalborg University Hospital, Aalborg, Denmark** (Prof K Overvad)

Correspondence to:
Dr Nicola P Bondonno, Medical Research Foundation, Perth, WA 6000, Australia
n.bondonno@ecu.edu.au

Research in context

Evidence before this study

We searched Google Scholar and PubMed for studies in any language published up to and including Jan 31, 2019 (with no earliest date specified), analysing the association between flavonoid intake and cardiovascular disease. We used the search terms "flavonoid", "polyphenol", "cardiovascular disease", "heart disease", and "stroke". In a 2015 meta-analysis of 15 prospective cohort studies, individuals with the highest flavonoid intakes had a 15% lower relative risk of coronary heart disease compared with that of individuals with the lowest intakes. Although evidence suggests that flavonoids afford protection against atherosclerosis, to our knowledge, no studies have investigated the association between flavonoid intake and all cardiovascular diseases with an atherosclerotic cause. Additionally, although hypothesised, previous studies have been underpowered to detect differing associations in at-risk subpopulations.

Added value of this study

Our study provides evidence of non-linear associations between total flavonoid intake and atherosclerotic cardiovascular disease, which plateau at intakes of approximately 1000 mg per day. To our knowledge, this is the first study to show that for smokers and consumers of high quantities of alcohol, the association between flavonoid intake and atherosclerotic cardiovascular disease was stronger and additional benefit was seen for higher intakes compared with that for non-smokers and individuals who consumed low-to-moderate amounts of alcohol. Furthermore, this is the first study to suggest that cardiovascular protective effects of flavonoids occur primarily in the peripheral arteries.

Implications of all the available evidence

These findings highlight a potential strategy to improve population health with dietary recommendations ensuring the adequate consumption of flavonoid-rich foods. Our findings suggest that flavonoids might have an even more crucial role in the prevention of atherosclerotic cardiovascular disease in high-risk populations.

suggests that flavonoids might ameliorate the initiation and progression of atherosclerosis, the leading cause of cardiovascular disease, through regulation of inflammation and redox processes.[8] Large long-term epidemiological studies are now needed to examine associations between flavonoid intake and atherosclerotic cardiovascular disease, particularly in subpopulations prone to atherosclerosis.[9]

See Online for appendix

Therefore, the primary aim of our study was to investigate the association of total flavonoid intake and intake by flavonoid subclass and of major flavonoid compounds with incident atherosclerotic cardiovascular disease in the Danish Diet, Cancer, and Health cohort. Secondary aims were to assess whether these associations differed by sex, smoking status, alcohol consumption, body-mass index (BMI), and presence of prevalent diabetes.

Methods

Study design and population

For our prospective cohort study, we used the Danish Diet, Cancer, and Health study, an associated cohort of the European Prospective Investigation into Nutrition and Cancer, which recruited residents of the greater areas of Copenhagen and Aarhus.[10] Eligible participants for this study were aged 50–65 years and had completed a food-frequency questionnaire at baseline in the Danish Diet, Cancer, and Health study. Using the Danish civil registration system, we linked participants to nationwide registers on the individual level. The following databases were cross-linked to the cohort: the Civil Registration System, the Integrated Database for Labor Market Research, and the Danish National Patient Register (DNPR). The DNPR holds information on date of hospital admissions in Denmark since 1978 and date of discharge with one primary diagnosis and one or more secondary diagnoses defined by the International Classification of Diseases (ICD), the 8th revision (ICD-8) until 1993 and the 10th revision (ICD-10) from 1994 onwards (appendix p 1).[11] Participants were excluded if they had any prevalent atherosclerotic cardiovascular disease, which included self-reported myocardial infarction, previous diagnosis of ischaemic heart disease, self-reported ischaemic stroke, previous diagnosis of ischaemic stroke, or previous diagnosis of peripheral artery disease. Furthermore, participants were excluded if they had missing covariates, were extreme outliers (appendix p 7), or had implausible energy intakes (<2092 kJ per day [<500 kcal per day] or >20 920 kJ per day [>5000 kcal per day]).

In Denmark, register studies do not require approval from an ethics committee. This study was approved by the Danish Data Protection Agency.

Procedures

Exposures in our study were intakes of total flavonoids, and flavonoid subclasses and individual flavonoid compounds with mean intakes greater than 5 mg per day. These intakes were estimated from dietary data, collected at baseline by use of a validated 192-item food-frequency questionnaire mailed out to participants before their visit to one of the two study centres.[12] Full details of the Phenol-Explorer calculations have been published previously.[13,14] Phenol-Explorer is a comprehensive database on the flavonoid and, more broadly, the polyphenol content of more than 400 foods.

	Total population (n=53 552)	Total flavonoid intake				
		Quintile 1 (n=10 711)	Quintile 2 (n=10 710)	Quintile 3 (n=10 710)	Quintile 4 (n=10 710)	Quintile 5 (n=10 711)
Total flavonoid intake (mg per day)	499 (290–808)	175 (129–215)	323 (290–360)	498 (445–552)	730 (663–808)	1205 (1028–1437)
Sex						
Men	24 952 (46·6%)	6069 (56·7%)	5392 (50·3%)	4999 (46·7%)	4644 (43·4%)	3848 (35·9%)
Women	28 600 (53·4%)	4642 (43·3%)	5318 (49·7%)	5711 (53·3%)	6066 (56·6%)	6863 (64·1%)
Age (years)	56 (52–60)	55 (52–60)	56 (52–60)	56 (52–60)	56 (52–60)	55 (52–59)
BMI (kg/m^2)	25·5 (23·2–28·1)	26·1 (23·7–28·8)	25·8 (23·6–28·4)	25·5 (23·3–28·2)	25·3 (23·1–27·8)	24·8 (22·7–27·3)
MET score	56·5 (37·0–84·8)	51·0 (32·5–78·0)	55·5 (36·5–84·0)	57·3 (38·5–84·7)	58·5 (38·8–87·0)	60·0 (40·0–88·5)
Smoking status						
Never	19 258 (36·0%)	2724 (25·4%)	3684 (34·4%)	3922 (36·6%)	4322 (40·4%)	4606 (43·0%)
Former	15 205 (28·4%)	2524 (23·6%)	2867 (26·8%)	3059 (28·6%)	3405 (31·8%)	3350 (31·3%)
Current	19 089 (35·6%)	5463 (51·0%)	4159 (38·8%)	3729 (34·8%)	2983 (27·9%)	2755 (25·7%)
Education (years)						
≤7	17 293 (32·3%)	4832 (45·1%)	3990 (37·3%)	3365 (31·4%)	2838 (26·5%)	2268 (21·2%)
8–10	24 836 (46·4%)	4718 (44·0%)	5083 (47·5%)	5158 (48·2%)	5083 (47·5%)	4794 (44·8%)
≥11	11 400 (21·3%)	1156 (10·8%)	1634 (15·3%)	2183 (20·4%)	2784 (26·0%)	3643 (34·0%)
Mean household income (DKK per year)						
≤394 700	13 044 (24·4%)	3122 (29·1%)	2569 (24·0%)	2550 (23·8%)	2422 (22·6%)	2381 (22·2%)
394 701–570 930	13 289 (24·8%)	3095 (28·9%)	2848 (26·6%)	2577 (24·1%)	2467 (23·0%)	2302 (21·5%)
570 931–758 297	13 500 (25·2%)	2827 (26·4%)	2924 (27·3%)	2765 (25·8%)	2520 (23·5%)	2464 (23·0%)
>758 297	13 719 (25·6%)	1667 (15·6%)	2369 (22·1%)	2818 (26·3%)	3301 (30·8%)	3564 (33·3%)
Hypertensive	8201 (15·3%)	1597 (14·9%)	1694 (15·8%)	1662 (15·5%)	1642 (15·3%)	1606 (15·0%)
Hypercholesterolaemic	3397 (6·3%)	696 (6·5%)	676 (6·3%)	703 (6·6%)	707 (6·6%)	615 (5·7%)
Comorbidities						
Diabetes	1000 (1·9%)	240 (2·2%)	189 (1·8%)	202 (1·9%)	180 (1·7%)	189 (1·8%)
Heart failure	91 (0·2%)	16 (0·1%)	23 (0·2%)	18 (0·2%)	17 (0·2%)	17 (0·2%)
Atrial fibrillation	209 (0·4%)	37 (0·3%)	45 (0·4%)	43 (0·4%)	36 (0·3%)	48 (0·4%)
COPD	751 (1·4%)	190 (1·8%)	160 (1·5%)	142 (1·3%)	139 (1·3%)	120 (1·1%)
CKD	178 (0·3%)	35 (0·3%)	28 (0·3%)	39 (0·4%)	40 (0·4%)	36 (0·3%)
Cancer	227 (0·4%)	48 (0·4%)	40 (0·4%)	55 (0·5%)	32 (0·3%)	52 (0·5%)
Medication use						
Insulin	331 (0·6%)	70 (0·7%)	57 (0·5%)	71 (0·7%)	69 (0·6%)	64 (0·6%)
Antihypertensive	6122 (11·4%)	1181 (11·0%)	1287 (12·0%)	1242 (11·6%)	1212 (11·3%)	1200 (11·2%)
Statin	624 (1·2%)	133 (1·2%)	138 (1·3%)	129 (1·2%)	122 (1·1%)	102 (1·0%)
HRT						
Never	15 553 (29·0%)	2560 (23·9%)	2976 (27·8%)	3163 (29·5%)	3204 (29·9%)	3650 (34·1%)
Current	8610 (16·1%)	1283 (12·0%)	1513 (14·1%)	1664 (15·5%)	1948 (18·2%)	2202 (20·6%)
Former	4406 (8·2%)	790 (7·4%)	825 (7·7%)	875 (8·2%)	910 (8·5%)	1006 (9·4%)
NSAID	17 081 (31·9%)	3268 (30·5%)	3312 (30·9%)	3444 (32·2%)	3433 (32·1%)	3624 (33·8%)
Aspirin	6484 (12·1%)	1233 (11·5%)	1254 (11·7%)	1318 (12·3%)	1280 (12·0%)	1399 (13·1%)
Dietary characteristics (g per day)						
Energy (kj)	9498 (7852–11 365)	8600 (7025–10 396)	9249 (7715–11 009)	9742 (8126–11 581)	9922 (8315–11 807)	9920 (8255–11 875)
Total fish intake	38 (25–55)	33 (22–48)	38 (25–54)	39 (27–57)	41 (28–59)	39 (27–56)
Red meat intake	78 (56–107)	80 (57–108)	81 (59–109)	80 (58–110)	78 (56–107)	72 (52–99)
Processed meat intake	24 (14–40)	28 (17–45)	26 (15–42)	25 (14–40)	23 (14–37)	20 (11–34)
Dietary fibre intake	20 (16–25)	17 (13–20)	19 (16–23)	21 (17–25)	22 (18–27)	23 (19–29)
Fruit intake	172 (95–282)	88 (45–142)	162 (98–239)	193 (114–301)	225 (140–360)	240 (141–390)
Vegetable intake	162 (105–231)	115 (72–171)	150 (100–212)	168 (114–236)	185 (127–254)	196 (136–272)
Alcohol intake	13 (6–31)	11 (3–23)	13 (6–25)	15 (6–33)	14 (7–32)	13 (6–32)

Data are median (IQR) or n (%), unless otherwise specified. BMI=body-mass index. MET=metabolic equivalent. DKK=Danish Krone. COPD=common obstructive pulmonary disease. CKD=chronic kidney disease. HRT=hormone replacement therapy. NSAID=non-steroidal anti-inflammatory drug.

Table 1: **Baseline characteristics**

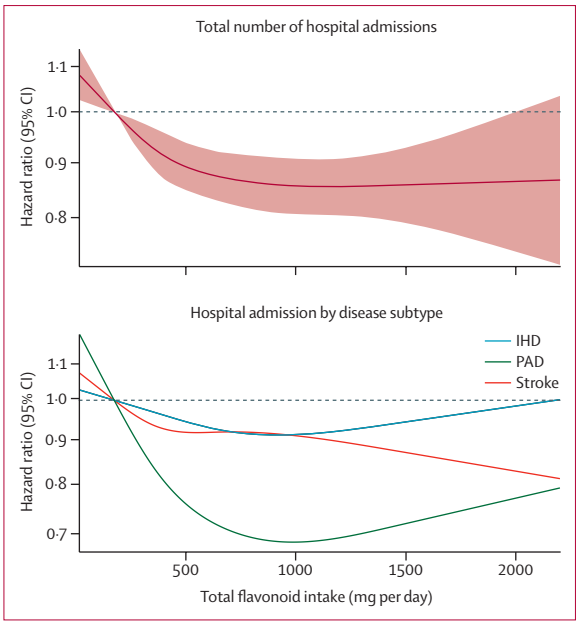

Figure 1: **Association between total flavonoid intake and hospital admissions for atherosclerotic cardiovascular disease**
Cubic spline curves describe the association between total flavonoid intake (mg per day) and hospital admissions for both total atherosclerotic cardiovascular disease and disease subtypes. Hazard ratios are based on Cox proportional hazards models adjusted for age, sex, body-mass index, smoking status, socioeconomic status (income), physical activity, and alcohol intake (model 2), and are comparing the specific level of flavonoid intake (horizontal axis) with the median intake for participants in the lowest intake quintile (175 mg per day). IHD=ischaemic heart disease. PAD=peripheral artery disease.

Significant correlations have been found between 24-h urine concentrations of 12 flavonoids and intake of their main food sources in 475 participants of the EPIC study.[15] In our study, we estimated the intakes of 219 different flavonoids. These were grouped into nine classes according to their chemical structure (flavonols, flavones, flavanols [flavanol monomers and flavanol oligomers and polymers], flavanones, isoflavones, anthocyanins, chalcones, dihydrochalcones, and dihydroflavonols) by summing the intakes of each individual flavonoid compound within their respective flavonoid class. We calculated total flavonoid intake by summing each of the flavonoid compounds.

We obtained information on sex, age, education, smoking habits, alcohol consumption, and daily activity from self-administered questionnaires completed by participants at Danish Diet, Cancer, and Health study enrolment. Dietary data were obtained from the semi-quantitative food frequency questionnaire. Anthropometric measures and total blood cholesterol were taken at the Danish Diet, Cancer, and Health study centres. We defined average annual income as household income after taxation and interest, for the value of the Danish currency in 2015, and we used the value over 5 years as a proxy for socioeconomic status. We used ICD-8 and ICD-10 codes for diagnosis of chronic kidney disease, chronic obstructive pulmonary disease, heart failure, atrial fibrillation, and cancers. For hypertension and diabetes, only self-reported data were used because of the low validity of ICD codes in DNPR.[16]

Outcomes

The primary outcome was a combined endpoint of first-time hospital admission for atherosclerotic cardiovascular disease. Atherosclerotic cardiovascular disease was defined as hospital admission with a primary or secondary diagnosis code for ischaemic heart disease, ischaemic stroke, or peripheral artery disease. Secondary outcomes were discrete hospital admissions for ischaemic heart disease, ischaemic stroke, and peripheral artery disease. These ICD codes have previously been validated in the DNPR.[16] Diagnoses of unstable angina or transient ischaemic attack were not included because these do not have validity and specificity in the DNPR.

To verify the registry-based outcomes, we re-examined associations using only medically reviewed and validated cases (appendix p 1).

Statistical analysis

Participants were followed up for a maximum of 23 years from the date of enrolment until death from any cause, emigration, hospital admission for atherosclerotic cardiovascular disease (first hospital admission for the combined endpoint), or end of follow-up (August, 2017), whichever came first. We computed Nelson-Aalen plots of cumulative incidence for atherosclerotic cardiovascular disease with a competing risk of death and by quintile of total flavonoid intake. We used restricted cubic splines with four knots (located at the fifth, 35th, 65th, and 95th percentiles) to investigate non-linear relationships between flavonoid intakes and all outcomes, with hazard ratios (HRs) derived from Cox proportional hazards models. We plotted HRs with 95% CIs for each unit of the exposure using the median intake in quintile 1 as the reference. Individuals with intakes greater than 4 SD above the mean were excluded from the spline analysis. The test of non-linearity used analysis of variance to compare the model only with the linear term to the model that included both linear and non-linear terms. We obtained HRs and 95% CIs for the median intakes in each quintile of the exposure variables from the splines. Cox proportional hazards assumptions were tested using log-log plots of the survival function versus time and assessed for parallel appearance, with no violation found. Because our aim was to obtain relative estimates for risk factors, all deaths were censored rather than treated as a competing risk. We used six models of adjustment: minimally adjusted (model 1), multivariable-adjusted (model 2), multivariable-adjusted including energy (model 3), multivariable-adjusted including covariates on the causal pathway (model 4), multivariable-adjusted including potential dietary

confounders (model 5), and multivariable-adjusted including potential dietary confounders that are also a source of flavonoids (model 6; appendix p 1). Covariates were chosen a priori according to the best of our knowledge of potential confounders of flavonoid intake and cardiovascular disease. We assumed absolute flavonoid intake to be more relevant than energy-adjusted values; therefore we did not include total energy intake as a covariate in the main models. However, energy intake was added to model 2 in a sensitivity analysis (model 3). Analyses were stratified by sex, BMI, baseline diabetes status, smoking status, and alcohol intake to test for potential effect modification. When stratifying by alcohol intake, we excluded all participants with an alcohol intake of zero (1180 participants) and when stratifying by BMI, we excluded those with a BMI lower than 18·5 (426 participants), because these were not our subgroups

	Quintile 1 (n=10 711)	Quintile 2 (n=10 710)	Quintile 3 (n=10 710)	Quintile 4 (n=10 710)	Quintile 5 (n=10 711)
Total flavonoids					
Number of events	2199	1844	1749	1573	1408
Intake (mg per day)*	175 (6–253)	323 (253–397)	498 (397–605)	730 (605–912)	1205 (912–3552)
HR (95% CI)					
Model 1	1 (ref)	0·84 (0·81–0·87)	0·74 (0·71–0·78)	0·70 (0·66–0·73)	0·66 (0·63–0·70)
Model 2	1 (ref)	0·94 (0·90–0·97)	0·89 (0·85–0·94)	0·87 (0·82–0·92)	0·85 (0·81–0·91)
Model 5	1 (ref)	0·95 (0·91–0·99)	0·91 (0·87–0·96)	0·90 (0·85–0·95)	0·89 (0·84–0·95)
Flavonols					
Number of events	2257	2581	2285	2194	2133
Intake (mg per day)*	15 (0–21)	26 (21–32)	39 (32–50)	66 (50–83)	116 (83–251)
HR (95% CI)					
Model 1	1 (ref)	0·83 (0·80–0·86)	0·73 (0·69–0·76)	0·67 (0·63–0·70)	0·65 (0·61–0·69)
Model 2	1 (ref)	0·92 (0·88–0·95)	0·86 (0·82–0·91)	0·85 (0·80–0·89)	0·84 (0·79–0·89)
Model 5	1 (ref)	0·92 (0·89–0·96)	0·88 (0·83–0·93)	0·87 (0·82–0·92)	0·87 (0·82–0·92)
Flavanol monomers					
Number of events	2279	1826	1722	1546	1400
Intake (mg per day)*	14 (0–21)	30 (21–46)	68 (46–116)	261 (116–282)	474 (282–916)
HR (95% CI)					
Model 1	1 (ref)	0·90 (0·88–0·92)	0·75 (0·71–0·80)	0·68 (0·64–0·72)	0·68 (0·64–0·72)
Model 2	1 (ref)	0·95 (0·93–0·97)	0·88 (0·83–0·93)	0·86 (0·82–0·92)	0·87 (0·82–0·92)
Model 5	1 (ref)	0·96 (0·94–0·98)	0·90 (0·85–0·95)	0·89 (0·84–0·94)	0·90 (0·84–0·95)
Flavanol oligo+polymers					
Number of events	2192	1934	1631	1578	1438
Intake (mg per day)*	93 (0–137)	180 (137–218)	257 (218–304)	361 (304–435)	538 (435–2254)
HR (95% CI)					
Model 1	1 (ref)	0·82 (0·78–0·85)	0·73 (0·70–0·77)	0·68 (0·64–0·71)	0·65 (0·62–0·69)
Model 2	1 (ref)	0·93 (0·89–0·97)	0·87 (0·83–0·92)	0·83 (0·78–0·88)	0·82 (0·78–0·87)
Model 5	1 (ref)	0·94 (0·90–0·98)	0·89 (0·85–0·94)	0·86 (0·81–0·90)	0·86 (0·81–0·91)
Anthocyanins					
Number of events	2043	1660	1597	1735	1738
Intake (mg per day)*	5 (0–9)	13 (9–17)	20 (17–24)	35 (24–53)	70 (53–397)
HR (95% CI)					
Model 1	1 (ref)	0·77 (0·73–0·80)	0·71 (0·67–0·75)	0·75 (0·71–0·79)	0·83 (0·78–0·88)
Model 2	1 (ref)	0·91 (0·87–0·96)	0·89 (0·84–0·95)	0·94 (0·88–0·99)	0·98 (0·92–1·05)
Model 5	1 (ref)	0·92 (0·88–0·97)	0·91 (0·85–0·97)	0·96 (0·90–1·02)	1·01 (0·94–1·07)
Flavanones					
Number of events	1939	1734	1761	1591	1748
Intake (mg per day)*	3 (0–6)	9 (6–13)	18 (13–26)	32 (26–50)	70 (50–364)
HR (95% CI)					
Model 1	1 (ref)	0·92 (0·88–0·96)	0·85 (0·80–0·91)	0·83 (0·79–0·88)	0·86 (0·81–0·91)
Model 2	1 (ref)	0·98 (0·94–1·02)	0·96 (0·90–1·02)	0·93 (0·88–0·99)	0·95 (0·90–1·01)
Model 5	1 (ref)	0·99 (0·95–1·03)	0·97 (0·91–1·03)	0·95 (0·89–1·00)	0·96 (0·91–1·02)

(Table 2 continues on next page)

	Quintile 1 (n=10 711)	Quintile 2 (n=10 710)	Quintile 3 (n=10 710)	Quintile 4 (n=10 710)	Quintile 5 (n=10 711)
(Continued from previous page)					
Flavones					
Number of events	1941	1809	1672	1644	1707
Intake (mg per day)*	2 (0–3)	4 (3–5)	5 (5–6)	7 (6–9)	11 (9–51)
HR (95% CI)					
Model 1	1 (ref)	0·84 (0·79–0·88)	0·80 (0·75–0·84)	0·77 (0·73–0·81)	0·79 (0·75–0·84)
Model 2	1 (ref)	0·93 (0·88–0·98)	0·91 (0·86–0·96)	0·89 (0·84–0·94)	0·90 (0·85–0·96)
Model 5	1 (ref)	0·94 (0·89–0·99)	0·93 (0·87–0·98)	0·91 (0·86–0·97)	0·94 (0·88–1·00)

Hazard ratios (HR; 95% CI) for hospital admissions for atherosclerotic cardiovascular disease during 23 years of follow-up, obtained from restricted cubic splines based on Cox proportional hazards models. Model 1 adjusted for age and sex; model 2 adjusted for age, sex, body-mass index, smoking status, physical activity, alcohol intake, and social economic status (income); model 5 adjusted for all covariates in model 2 plus intakes of fish, red meat, processed food, polyunsaturated fatty acids, monounsaturated fatty acids, and saturated fatty acids. *Median (range).

Table 2: **Hospital admissions for atherosclerotic cardiovascular disease by quintiles of flavonoid intake**

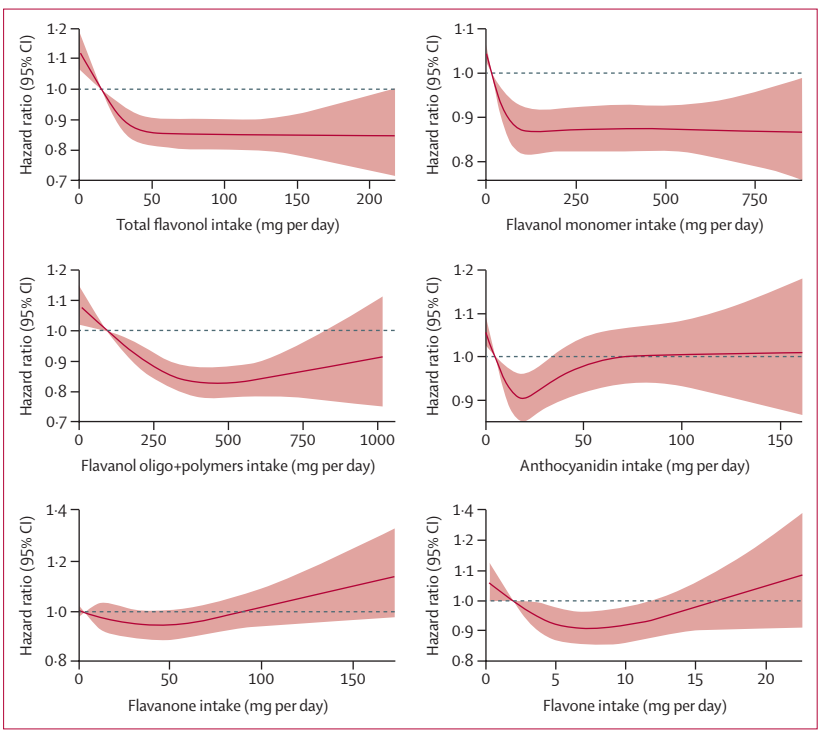

Figure 2: **Association between flavonoid subclass intakes (mg per day) and total hospital admissions for atherosclerotic cardiovascular disease**

Hazard ratios are based on Cox proportional hazards models adjusted for age, sex, body-mass index, smoking status, social economic status (income), physical activity, and alcohol intake (model 2), and are comparing the specific level of flavonoid intake (horizontal axis) with the median intake for participants in the lowest intake quintile.

of interest. We chose stratification cutoff points of 20 g pure alcohol per day (two standard drinks) and a BMI of 30 kg/m², because the risk of mortality is higher beyond these levels.[17] We did analyses using STATA/IC, version 14.2, and R statistics. Statistical significance was set at p≤0·05 (two-tailed) for all tests.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. FD, NPB, and GG had full access to all the data in the study. All authors had final responsibility for the decision to submit for publication.

Results

Between Nov 24, 1993, and May 28, 1997, the Danish Diet, Cancer, and Health study recruited 57 053 participants. Of these, 56 468 completed a food-frequency questionnaire at baseline. 2916 participants did not meet the inclusion criteria, resulting in a study population of 53 552 participants (appendix p 2).

Our study cohort had a median age of 56 years (IQR 52–60) at entry and a median follow-up of 21 years (15–22). During a total of 939 031 person-years of follow-up, 8773 individuals were admitted to hospital for any atherosclerotic cardiovascular disease, 5323 for ischaemic heart disease, 2920 for ischaemic stroke, and 1867 for peripheral artery disease. Furthermore, 9522 participants died from any cause without having been admitted to hospital for an atherosclerotic cardiovascular disease. The cumulative incidence of hospital admissions for atherosclerotic cardiovascular disease and death without admission for atherosclerotic cardiovascular disease are shown in the appendix (p 3).

Participants in the highest quintile of flavonoid intake were more likely to be women and tended to have a lower BMI; be more physically active; have never smoked; have a higher education degree; have a higher income; eat more fish, fibre, vegetables, and fruit; and eat less red and processed meat than participants in the lowest quintile of flavonoid intake (table 1).

Cumulative incidences of atherosclerotic cardiovascular disease, ischaemic heart disease, ischaemic stroke, and peripheral artery disease for each quintile of total flavonoid intake are shown in the appendix (p 3). The inverse association between total flavonoid intake and total atherosclerotic cardiovascular disease was non-linear (p<0·001), and the restricted cubic splines showed a threshold of association at approximately 1000 mg per day (figure 1). Compared with a total flavonoid intake of

175 mg per day (median intake in quintile 1), an intake of 1000 mg per day was associated with a 14% lower risk of atherosclerotic cardiovascular disease (HR 0·86, 95% CI 0·81–0·91). For atherosclerotic cardiovascular disease subtypes, a total flavonoid intake of 1000 mg per day was associated with a 9% lower risk of ischaemic heart disease (0·91, 0·85–0·98), a non-significant 9% lower risk of ischaemic stroke (0·91, 0·82–1·01), and a 32% lower risk of peripheral artery disease (0·68, 0·60–0·78) compared with a 175 mg per day intake (model 2). For all flavonoid subclasses, after multivariable adjustments (models 2 and 3), participants in quintiles 2–5 had a lower risk of atherosclerotic cardiovascular disease than that of participants in quintile 1 (table 2). HRs for alternative models of adjustment (models 4 and 6) did not differ substantively from HRs from model 2 (appendix p 4). We observed thresholds of benefit at varying levels of intake for each flavonoid subclass (figure 2).

Intakes of kaempferol, quercetin, epicatechin, procyanidin dimers, and apigenin greater than those in quintile 1 were associated with a significantly lower risk of hospital admissions for atherosclerotic cardiovascular disease after adjustment for potential lifestyle (model 2) and dietary confounders (model 5), with no further differences in HRs observed beyond quintile 4 (figure 3, appendix pp 5–6). The observed association was more linear for malvidin and procyanidin trimers than for other compounds. The HRs were 0·76 (95% CI 0·70–0·83) for malvidin and 0·79 (0·74–0·84) for procyanidin trimers in quintile 5 versus quintile 1 after adjustment for potential lifestyle confounders (model 2).

The association between flavonoid intake and atherosclerotic cardiovascular disease differed according to smoking status, alcohol intake, and BMI, but not by prevalence of diabetes or by sex for intakes lower than 1000 mg per day.

Because we observed no difference in the association between flavonoid intake and hospital admissions for atherosclerotic cardiovascular disease for never smokers compared with that for former smokers, these subgroups were combined (data not shown). The inverse association between total flavonoid intake and atherosclerotic cardiovascular disease was stronger in smokers than in non-smokers and stronger in participants who consumed more than 20 g per day of alcohol than in those who consumed 20 g per day or less of alcohol (figure 4). A total flavonoid intake of 1000 mg per day was associated with a 22% lower risk of atherosclerotic cardiovascular disease in smokers (HR 0·78, 95% CI 0·72–0·85), a non-significant 5% lower risk in non-smokers (0·95, 0·87–1·03), a 21% lower risk in high alcohol consumers (0·79, 0·71–0·87), and an 11% lower risk in low-to-moderate alcohol consumers (0·89, 0·82–0·95; model 2). Comparing participants in quintile 5 with those in quintile 1 of total flavonoid intake, the HRs for hospital admissions for atherosclerotic cardiovascular disease

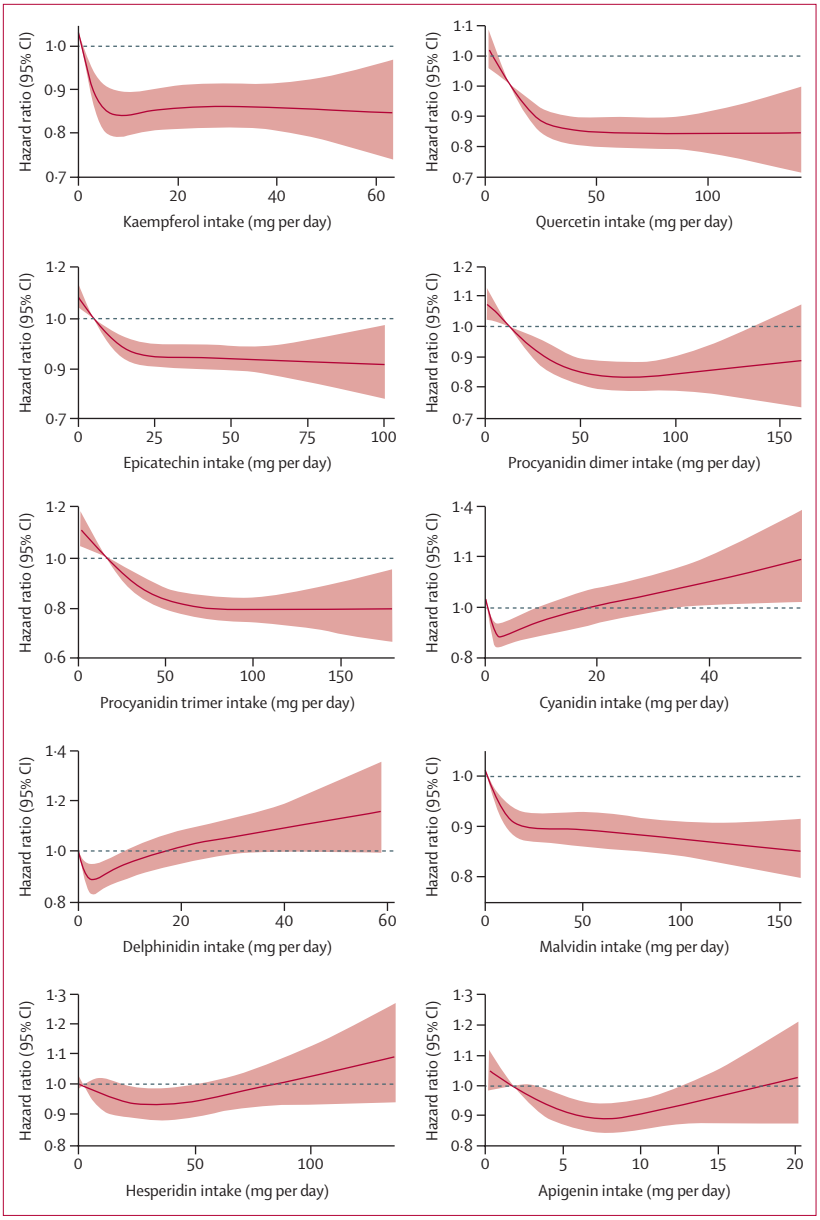

Figure 3: **Association between major flavonoid compound intakes (mg per day) and total hospital admissions for atherosclerotic cardiovascular disease**

Hazard ratios are based on Cox proportional hazards models adjusted for age, sex, body-mass index, smoking status, social economic status (income), physical activity, and alcohol intake (model 2), and are comparing the specific level of flavonoid intake (horizontal axis) with the median intake for participants in the lowest intake quintile.

lowered with increasing smoking intensity (pack-years) and were lowest for participants who consumed 20–40 g per day of alcohol (appendix p 6). We observed a lower risk of atherosclerotic cardiovascular disease associated with higher flavonoid intake in participants with normal weight and overweight (BMI 18·5–30), but not in those who were obese (BMI >30; figure 4, appendix p 6). A total flavonoid intake of 1000 mg per day was associated with a 20% lower risk of atherosclerotic cardiovascular disease in participants with BMI 18·5–30 (HR 0·80, 95% CI

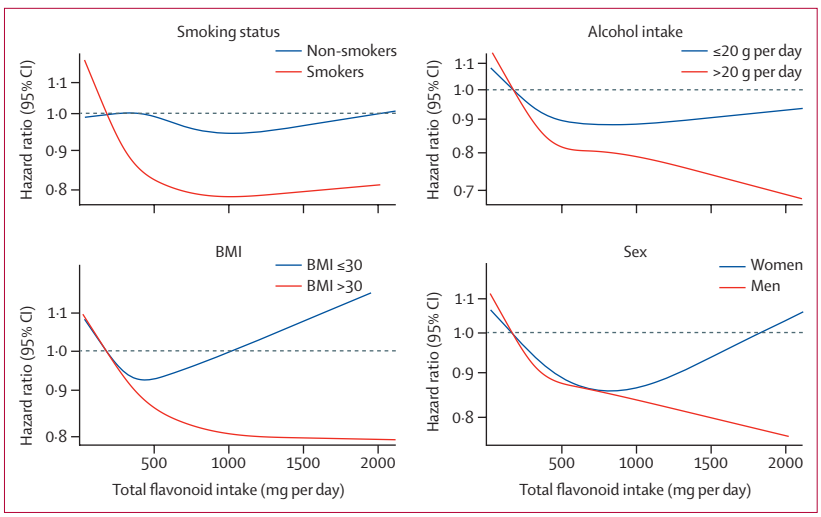

Figure 4: **Stratified, multivariable-adjusted associations between total flavonoid intake and total hospital admissions for atherosclerotic cardiovascular disease**

Multivariable-adjusted associations are stratified by baseline smoking status, alcohol intake, BMI, and sex. Hazard ratios are based on Cox proportional hazards models and are comparing the specific level of flavonoid intake (horizontal axis) with the median intake for participants in the lowest intake quintile (175 mg per day). All analyses were standardised for age, sex, BMI, smoking status, social economic status (income), physical activity, and alcohol intake (model 2), not including the stratification variable for each subgroup. BMI=body-mass index.

0·75–0·86) than an intake of 175 mg per day, but no lower risk in participants with a BMI higher than 30 (HR 0·99, 0·87–1·14). A difference in association between men and women was apparent only for intakes greater than 1000 mg per day, in which 95% CIs also widened substantially.

Using only validated cases, and after excluding a further seven participants who had a diagnosis of validated atherosclerotic cardiovascular disease (n=53 545), 3479 participants were admitted to hospital for any atherosclerotic cardiovascular disease, 2505 were admitted for myocardial infarction, 923 for ischaemic stroke, and 818 for peripheral artery disease. Compared with a total flavonoid intake of 175 mg per day (median intake in quintile 1), an intake of 1000 mg per day was associated with a 17% lower risk of atherosclerotic cardiovascular disease (HR 0·83, 95% CI 0·76–0·91). For atherosclerotic cardiovascular disease subtypes, a total flavonoid intake of 1000 mg per day, compared with the median intake in quintile 1, was associated with a non-significant 9% lower risk of myocardial infarction (0·91, 0·82–1·02), an 18% lower risk of ischaemic stroke (0·82, 0·68–0·98), and a 35% lower risk of peripheral artery disease (0·65, 0·53–0·79; appendix p 7).

Discussion

Diet is a primary modifiable risk factor for cardiovascular disease.[2] Therefore, dietary changes have great potential to affect cardiovascular disease incidence, especially in populations at high risk. In this prospective cohort study of 53 552 Danish people without atherosclerotic cardiovascular disease at recruitment, we found that a moderate intake of flavonoids was inversely associated with incident hospital admissions for atherosclerotic cardiovascular disease, with no additional benefits seen for intakes greater than 1000 mg per day. This inverse association was strongest in subpopulations prone to atherosclerosis including smokers and individuals who consumed high quantities of alcohol.

Flavonoid intake in Denmark is similar to intakes reported for other European countries and generally lower than intakes reported for the UK, because of a lower average consumption of tea.[13] In the past few years, studies assessing the relationship between flavonoid intake and cardiovascular disease have focused primarily on mortality from any cardiovascular disease[18] or coronary heart disease and ischaemic stroke, both individually and combined.[19,20] A 2017 meta-analysis found that total flavonoid intake was marginally, but not significantly, associated with a lower risk of cardiovascular disease mortality (relative risk 0·85, 95% CI 0·70–1·03) for the highest versus lowest category of total flavonoid intake;[18] similar risk estimates have been reported in other meta-analyses.[21,22] Indeed, our previous study in the Danish Diet Cancer, and Health cohort showed that participants in the highest quintile of flavonoid intake had a 15% lower risk of cardiovascular disease mortality (HR 0·85, 95% CI 0·78–0·93) than that of participants in the first quintile.[23] Although a trend for a reduced risk of cardiovascular disease with higher levels of flavonoid intake exists, this is generally non-significant after adjusting for potential confounders.[18] In this study, we used hospital admission for ischaemic heart disease, ischaemic stroke, and peripheral artery disease as markers of atherosclerotic and thromboembolic cardiovascular disease rather than an unselective array of cardiovascular disease diagnoses. These events were our chosen outcomes of interest because in-vivo evidence suggests that flavonoids might impede the initiation and progression of atherosclerosis by restoring endothelial homoeostasis and attenuating inflammation.[7] In our study, total flavonoid intake was significantly associated with less hospital admissions for atherosclerotic cardiovascular disease after adjustment for potential lifestyle and dietary confounders. This result might be due to our use of hospital admissions rather than mortality, allowing us to include peripheral artery disease, which is an underlying risk factor for cardiovascular disease mortality and a marker of widespread atherosclerosis, but is rarely fatal in itself.[24] To our knowledge, one other observational study (n=1658) has looked at combined cardiovascular disease incidence, including peripheral artery disease, and reported an inverse association with total flavonoid intake when comparing the highest tertile with the lowest (HR 0·46, 95% CI 0·28–0·75).[25] In our study, the inverse association between total flavonoid and atherosclerotic cardiovascular disease plateaued at intakes of approximately 1000 mg per day. Two cups of black tea,

100 g of blueberries, one apple, one glass of red wine, 100 g of black beans, and 100 g of dark chocolate would provide more than 1000 mg of flavonoids.[4] Evidence exists that flavonoid bioactivity does not follow a classic linear dose–response association.[20] However, the threshold might differ in specific subpopulations at elevated cardiovascular disease risk, such as smokers and individuals who consume high quantities of alcohol.

Flavonoids have been reported to positively influence cardiovascular disease by acting upon common cross-talking proteins that control the anti-inflammatory Nrf2 pathway and the pro-inflammatory NF-$_{\kappa}\beta$ pathway.[7] Additionally, after metabolism, flavonoid conjugates in the circulatory system can act on both endothelial cells and underlying smooth muscle cells, regulating vascular health[7] We provide hypothesis-stimulating evidence that the inverse associations observed for flavonoid intake were strongest for peripheral artery disease, followed by ischaemic stroke and ischaemic heart disease. This might be due to the underlying pathology of these diseases and the mechanisms by which flavonoids exert their cardiovascular protective effects, which our results suggest are primarily in the peripheral arteries and should be investigated further.

Results from our study indicate that the inverse association between flavonoid intake and atherosclerotic cardiovascular disease was stronger in current smokers and individuals who consume high quantities of alcohol, two populations at a higher risk of atherosclerosis and clinical atherosclerotic cardiovascular disease than those who do not smoke or drink or do so in low quantities. Cigarette smoking is an important risk factor for atherosclerosis and peripheral artery disease.[26] In a randomised controlled trial done in healthy smokers, supplementation with flavonoid-rich grape juice restored vascular function and decreased smoking-induced inflammatory and fibrinolytic biomarkers.[27] Taken together, these findings provide evidence that flavonoid-rich foods might mitigate the risk of atherosclerotic cardiovascular disease in smokers. The finding that the inverse association was stronger in individuals with high alcohol consumption is complex because red wine, a dietary source of flavonoids, is linked with cardiovascular health benefits.[28] Drinking more than two standard drinks per day has been consistently associated with an elevated risk of cardiovascular disease by a plethora of purported mechanisms, including an increase in blood pressure, systemic atherosclerosis, and inflammatory endothelial markers.[29] The increasing evidence that flavonoids might ameliorate the detrimental effects of cigarette smoking and high alcohol consumption[30,31] is supported by our study.

The weaker inverse association observed between flavonoid intake and atherosclerotic cardiovascular disease in participants with obesity opposes our a-priori hypothesis that flavonoids would be more protective in this subgroup, because this population is prone to higher levels of atherosclerosis than those of individuals with lower BMI. Although evidence exists that flavonoids affect body composition,[32] individuals with obesity have a disparate gut microbial composition.[33] This is important, because the gut microbiome has a crucial role in flavonoid metabolism and thus, in bioactivity,[34] which might explain the weak association in this subpopulation. We should note the differing baseline hazards between obese and non-obese subpopulations, indicating that these findings require corroboration on an absolute scale. With few people with diabetes at baseline, our study was probably underpowered to detect a difference in association between flavonoid intake and atherosclerotic cardiovascular disease in this high-risk population. Future research should assess if flavonoid intake is associated with atherosclerotic cardiovascular disease incidence in people with diabetes.

Our study has several strengths and some limitations. The large adult population followed up for 23 years, with a large number of events and almost no loss to follow-up, allowed us to examine associations in subpopulations. Our findings were supported, and even strengthened, with the validated case analysis. Because of the nature of the study, we were not able to infer causality or rule out residual or unmeasured confounding; however, the associations were not altered substantially by further adjustments. We acknowledge the limitations of common food-frequency questionnaires, particularly that not all flavonoid-rich foods (eg, berries) were included in the questionnaire. Because we only had baseline data, we acknowledge that some of these measures might have changed over the 23 years of follow-up; however, this measurement error would have most likely attenuated any associations. Furthermore, because the Danish population is more homogeneous than many other countries, further studies are warranted to determine whether similar associations exist in populations outside of this cohort.

In this Danish prospective cohort study, we observed an inverse association between flavonoid intake of up to 1000 mg per day and hospital admissions for atherosclerotic cardiovascular disease, after which we detected no additional benefits. The strongest associations observed were for peripheral artery disease and for atherosclerotic cardiovascular disease in smokers and high alcohol consumers. These findings need to be further explored and, if replicated, might provide a novel means of mitigating atherosclerotic cardiovascular disease risk by ensuring an adequate consumption of flavonoid-rich foods, particularly in subpopulations prone to atherosclerosis.

Contributors

NPB, FD, KO, AT, and JMH contributed to the study concept and design. AS calculated the flavonoid intake from food frequency questionnaire data. NPB and FD did the data analysis. NPB and FD drafted the manuscript. All authors critically reviewed the final draft of the manuscript.

Declaration of interests
We declare no competing interests.

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