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Flavonoid intakes inversely associate with COPD in smokers

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Shareable abstract (@ERSpublications)
While smoking cessation should remain the top priority for COPD prevention, the findings from this study suggest that dietary flavonoids are important in partially mitigating the risk of COPD in people who smoke or who used to smoke https://bit.ly/3r6wWt8

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Abstract

Introduction Higher flavonoid intakes are beneficially associated with pulmonary function parameters; however, their association with chronic obstructive pulmonary disease (COPD) is unknown. This study aimed to examine associations between intakes of 1) total flavonoids, 2) flavonoid subclasses and 3) major flavonoid compounds with incident COPD in participants from the Danish Diet, Cancer and Health study.

Methods This prospective cohort included 55 413 men and women without COPD, aged 50–65 years at recruitment. Habitual flavonoid intakes at baseline were estimated from a food frequency questionnaire using Phenol-Explorer. Danish nationwide registers were used to identify incident cases of COPD. Associations were modelled using restricted cubic splines within Cox proportional hazards models.

Results During 23 years of follow-up, 5557 participants were diagnosed with COPD. Of these, 4013 were current smokers, 1062 were former smokers and 482 were never-smokers. After multivariable adjustments, participants with the highest total flavonoid intakes had a 20% lower risk of COPD than those with the lowest intakes (quintile 5 *versus* quintile 1: HR 0.80, 95% CI 0.74–0.87); a 6–22% lower risk was observed for each flavonoid subclass. The inverse association between total flavonoid intake and COPD was present in both men and women but only in current smokers (HR 0.77, 95% CI 0.70–0.84) and former smokers (HR 0.82, 95% CI 0.69–0.97), not never-smokers. Furthermore, higher flavonoid intakes appeared to lessen, but not negate, the higher risk of COPD associated with smoking intensity.

Conclusion Dietary flavonoids may be important for partially mitigating the risk of smoking-related COPD. However, smoking cessation should remain the highest priority.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common lung condition characterised by persistent respiratory symptoms and irreversible airflow limitations due to an abnormal inflammatory response of the lungs, usually caused by significant exposure to noxious gases or particles [1, 2]. While smoking is the most common cause of COPD, other modifiable risk factors include air pollution; occupational exposure to dust, vapours and fumes; and respiratory infections in early life [3]. Non-modifiable risk factors include

age and genetic predisposition [4]. Given the high prevalence and burden of COPD worldwide, and that there is currently no cure [5], prevention strategies should be prioritised.

There is emerging evidence that diet has an impact on lung function and may play a protective role against COPD [6–8], likely through the modulation of inflammation and oxidative stress pathways that are implicated in the development of this chronic disease [9]. In a prospective cohort study of Swedish men, a strong inverse association between total fruit and vegetable consumption and COPD was observed in current and former smokers but not in never-smokers [10]. Fruit and vegetables, as well as tea, cocoa and other plant-based foods and beverages, are dietary sources of flavonoids, which are bioactive compounds that reduce oxidative stress and systematic inflammation [11]. Flavonoids are a class of polyphenols, and can be further categorised into subclasses based on their chemical structure. Although epidemiological studies investigating the association between flavonoid intakes specifically and COPD are missing, there is some evidence that flavonoid intakes are favourably associated with pulmonary function parameters [12, 13] and less age-related decline in lung function [14].

The primary aim of this study was to investigate the associations between intakes of 1) total flavonoids, 2) flavonoid subclasses and 3) key flavonoid compounds within respective subclasses with incident COPD in the Danish Diet, Cancer and Health cohort. In our previous work, a consistent finding was that associations between flavonoid intake and a range of chronic diseases (including cardiovascular disease, dementia and diabetes [15, 16]) were stronger in current and former smokers. Given the importance of smoking as a risk factor for COPD, the purported antioxidant and anti-inflammatory properties of flavonoids, and that there is evidence of sex-related differences in COPD risk and outcomes [17], secondary aims were to explore interactions between flavonoid intakes and risk factors for COPD, namely sex and smoking.

Methods

Study population

The present study was conducted using data collected from participants of the Danish Diet, Cancer and Health study. A detailed description of the original study has been published previously [18]. In brief, 57 053 participants were recruited from the cities of Copenhagen and Aarhus in Denmark between 1993 and 1997. Upon enrolment, 56 468 of these participants completed a food frequency questionnaire (FFQ) and had not been diagnosed with cancer. Data collected were cross-linked to the following nationwide registers, using the unique and permanent civil registration number assigned to all Danish residents: the Civil Registration System, the Integrated Database for Labor Market Research and the Danish National Patient Register. The latter register [19] holds information on all hospital admissions and visits to outpatient clinics and urgent care centres in Denmark since 1978. It includes one primary diagnosis and one or more secondary diagnoses defined by the International Classification of Diseases (ICD) (8th revision (ICD-8) until 1993 and 10th revision (ICD-10) from 1994 to present). In the present study, participants were excluded if they had a diagnosis of COPD, unspecified chronic bronchitis or emphysema prior to enrolment into the Danish Diet, Cancer and Health study (n=479; ICD-8: 491.00–492.09, ICD-10: J42–J44), if they had reported improbable energy intakes (n=202; <2092 kJ per day (<500 kcal per day) and >20 920 kJ per day (>5000 kcal per day)) or if they had missing or extreme values for covariates (n=374; supplementary figure E1).

This study was approved by the Danish Data Protection Agency (ref no. 2012–58-0004 I-Suite nr: 6357, VD-2018-117).

Exposures

Primary exposures of interest in the present study were total flavonoid intake (calculated by summing intakes of each of the 219 flavonoid compounds) and intakes of flavonoid subclasses and individual flavonoid compounds, with mean intakes >5 mg per day at baseline. A detailed description of how flavonoid intakes were estimated from the FFQ using the Phenol-Explorer database [20] has been published previously [15]. In brief, flavonoid estimates (mg per 100 g fresh food weight) for each food and beverage in the FFQ (n=174) were derived from the Phenol-Explorer database taking into consideration food processing using retention factors. These were then multiplied by intakes of respective foods and beverages (g per day). In a *post hoc* investigative analysis, smoking pack-years (lifetime average number of cigarettes smoked multiplied by the number of years smoked divided by 20) was modelled as the exposure of interest.

Study outcomes

The primary outcome was a first-time hospitalisation or outpatient visit with a primary or secondary diagnosis of COPD (ICD-10: J44), unspecified chronic bronchitis (ICD-10: J42) or emphysema (ICD-10: J43). The ICD-10 code J44 has previously been validated in the Danish National Patient Register and has a positive predictive value of 92% (95% CI 91–93%) [21]. Because patients with COPD may have been coded as having unspecified chronic bronchitis (ICD-10: J42) or emphysema (ICD-10: J43), these were also included. A first-time diagnosis of COPD is the only validated method to identify COPD in Danish registers; hereinafter this will be referred to as incident COPD. Most cases were identified by ICD-10 codes DJ449 (~69%), DJ441 (~12%) and DJ429 (~8%).

Covariates

A description of the covariates used is given in the supplementary material and supplementary table E1.

Statistical analysis

Multivariable Cox proportional hazards models were used to investigate relationships between self-reported flavonoid intake and incident COPD. Each participants' time-to-event was calculated from the date of enrolment into the Danish Diet, Cancer and Health study up until the date of a first-time diagnosis of COPD, death, emigration from Denmark or the end of follow-up (August 2017), whichever came first. Cox proportional hazards assumptions were tested by plotting Schoenfeld residuals, with no violation found. To allow the association between all continuous covariates, including the exposures of interest, and outcome to be non-linear, these variables were modelled with restricted cubic splines using the "rms" R package with the rcs() function [22] in R (www.r-project.org). Quintiles (Q1–5) of each flavonoid exposure variable were generated, and the median value of each quintile was calculated. Hazard ratios, obtained from the Cox proportional hazards models described above, are relative to the median flavonoid intake in Q1 (reference value); these were plotted against the exposure variable, with 95% confidence interval bands provided. In addition, hazard ratios and 95% confidence intervals were calculated from the fitted models, comparing the median of each quintile to the reference value of the median in Q1, and tabulated. For simpler visualisation, figures only include individuals with intakes $\leqslant$3 SD above the mean. Three models of adjustment were used: 1) minimally adjusted: age (years) and sex; 1b) multivariable-adjusted: age, sex, body mass index (BMI), smoking status (current/former/never), smoking pack-years, physical activity (total daily metabolic equivalent), pure alcohol intake (g per day), education ($\leqslant$7 years/8–10 years/$\geqslant$11 years) and socioeconomic status (income); 2) multivariable-adjusted including potential dietary confounders: all variables in Model 1b plus energy intake (kJ per day) and intakes (g per day) of fish, red meat, processed meat, whole grains, refined grains, polyunsaturated fatty acids, monounsaturated fatty acids and saturated fatty acids. Confounders were selected based on *a priori* knowledge [23–25].

A secondary aim was to investigate interactions with established risk factors for COPD [3] for which data had been collected at baseline (specifically, smoking status and sex). First, analyses were stratified by sex and smoking status to examine the consistency of the associations. Because there is potential for residual confounding by smoking intensity, smoking pack-years was included as a covariate in the model when stratifying by smoking status. Interaction on the multiplicative scale was assessed by likelihood ratio tests of Cox proportional hazards models with and without the interaction terms. Second, standard logistic regression models were used to obtain the 20-year absolute risk estimates of a healthcare visit for COPD. These analyses used a binary outcome designating the occurrence of a COPD healthcare visit during the first 20 years of follow-up. Unless indicated by the relevant stratification variable, these estimates are for the "average" smoking cohort participant, *i.e.* a smoker, aged 56 years, with a BMI of 25.5 $kg \cdot m^{-2}$, a total daily metabolic equivalent score of 56, a mean household income of 394 701–570 930 DKK per year and an alcohol intake of 13 g per day. In a *post hoc* investigative analysis, we explored whether flavonoid intake modified the association between smoking intensity (represented by pack-years) and COPD, by plotting the predicted risk of COPD after 20 years of follow-up against pack-years, separately for men and women in the highest and lowest flavonoid intake quintiles. For the aforementioned analysis, smoking pack-years was entered in the model as a restricted cubic spline. Finally, to avoid the potential for preclinical COPD leading to reverse causation, as a sensitivity analysis we omitted all cases that occurred within the first 5 years of follow-up. All analyses were undertaken using STATA/IC 14.2 (StataCorp LLC) and R statistics (www.r-project.org).

Results

In this population of 55 413 Danish residents with a median age of 56 years, 5557 incident cases of COPD were identified during a maximum of 23 years of follow-up (median (IQR) follow-up: 21 years (19–22 years)). Furthermore, 11 195 participants died without a prior hospital diagnosis of COPD and 276 (~0.5%) were lost to follow-up.

Baseline characteristics

Overall, the cohort reported a daily median (IQR) habitual flavonoid intake of 496 mg (287–805 mg). Participants with higher total flavonoid intakes tended to be female and were more likely to be non-smokers, have a lower BMI, exercise more and have a higher education and income. Those consuming more flavonoids also ate, on average, more fish, whole grains, fruits and vegetables, and less red and processed meat (table 1).

Associations of total and flavonoid subclass intakes with incident COPD

The inverse association between total flavonoid intake and incident COPD was non-linear ($p_{non-linearity}<0.001$); the steepness of the slope decreased as flavonoid intakes increased (figure 1). Compared to participants in Q1, participants in Q5 had a 20% lower risk of COPD (HR 0.80, 95% CI 0.74–0.87) after multivariable adjustments (Model 1b, table 2). Non-linear associations were also observed for all flavonoid subclasses ($p_{non-linearity}<0.001$ for all, figure 1). Comparing high (Q4 or Q5) to low (Q1) intakes, the risk of COPD was up to 18% lower for flavonols, 14% lower for flavanol monomers, 22% lower for flavanol oligo+polymers, 12% lower for anthocyanins, 9% lower for flavanones and 15% lower for flavones, after multivariable adjustments (Model 1b, table 2). Significant associations were apparent for total flavonoid intakes (Q5 *versus* Q1: HR 0.85, 95% CI 0.78–0.92) and intakes of all flavonoid subclasses when potential dietary confounders were included as predictors in the model (Model 2, table 2).

Associations between major flavonoid compound intakes and incident COPD

Non-linear associations with incident COPD were observed for intakes of all major flavonoid compounds ($p_{non-linearity}<0.001$ for all, figure 2). Clear plateaus in the association were seen for intakes of kaempferol, quercetin, epicatechin, hesperidin and apigenin. Associations appeared to be more linear for intakes of malvidin and both proanthocyanidin dimers and trimers, and somewhat "u-shaped" for intakes of cyanidin and delphinidin. The lowest hazard ratios observed were for participants with the highest intakes of malvidin (Q5 *versus* Q1: HR 0.63, 95% CI 0.57–0.70; Model 1b; supplementary table E2).

Association between total flavonoid intake and incident COPD stratified by sex and smoking

Of the 5557 participants who were diagnosed with COPD during follow-up, 2605 were male and 2952 were female. The association between total flavonoid intake and incident COPD was present in both men and women although the shapes of the associations differed; the association for women was initially steeper, plateauing at a total flavonoid intake of ~500 mg per day ($p_{non-linearity}=0.013$), while the association for men was more linear ($p_{non-linearity}=0.506$, $p_{interaction}<0.001$, figure 3). On an absolute scale, women had a higher risk of COPD than men irrespective of their smoking status or flavonoid intake (table 3).

Of the 5557 COPD events, 4013 were in current smokers, 1062 were in former smokers, and 482 were in never-smokers. While a clear inverse association between total flavonoid intake and incident COPD was present in current/former smokers (termed ever-smokers), there was no inverse association in those who had never smoked ($p_{interaction}=0.017$, figure 3). Current and former smokers with the highest total flavonoid intakes had a 23% and 18% lower risk of COPD, respectively (current smokers Q5 *versus* Q1: HR 0.77, 95% CI 0.70–0.84; former smokers Q5 *versus* Q1: HR 0.82, 95% CI 0.69–0.97) after multivariable adjustments (Model 1b, supplementary tables E3 and E4). Hazard ratios for other flavonoid subclasses and compounds among current and former smokers only are presented in supplementary tables E3–E6. On an absolute scale, current and former smokers had a substantially higher risk of COPD than non-smokers irrespective of their flavonoid intake, although current smokers with a low flavonoid intake were at the highest risk (table 3). For participants who were current smokers at baseline, the difference in the 20-year predicted risk of COPD between a participant in the highest *versus* the lowest flavonoid intake quintile was 5.21% for men and 6.20% for women (table 3). Furthermore, for both men and women, flavonoid intakes appeared to modify the association between smoking intensity (pack-years) and predicted risk of COPD at 20 years in that participants with the highest flavonoid intakes (Q5) had a lower predicted risk of COPD than their low flavonoid-consuming (Q1) counterparts (figure 4).

Sensitivity analysis

Excluding events within the first 5 years of follow-up did not materially alter the observed associations (supplementary table E7).

Discussion

In this 23-year prospective cohort study of 55 413 Danish residents, we observed that baseline intakes of each flavonoid subclass, and all major flavonoid compounds investigated, were non-linearly inversely associated with incident COPD. The association between total flavonoid intake and incident COPD was only present in current and former smokers, who accounted for 91% of the COPD events observed. In the

TABLE 1 Baseline characteristics of study population

	Total population	Total flavonoid intake quintiles				
		Q1	Q2	Q3	Q4	Q5
Subjects, n	55 413	11 083	11 083	11 082	11 083	11 082
Total flavonoid intake (mg per day)	496 (287–805)	174 (127–213)	321 (287–357)	496 (442–549)	727 (660–805)	1203 (1025–1436)
Sex (male)	26 352 (47.6)	6410 (57.8)	5669 (51.2)	5279 (47.6)	4923 (44.4)	4071 (36.7)
Age (years)	56 (52–60)	56 (52–60)	56 (52–60)	56 (52–60)	56 (52–60)	55 (52–60)
BMI ($kg \cdot m^{-2}$)	25.5 (23.3–28.2)	26.1 (23.8–28.9)	25.9 (23.6–28.5)	25.6 (23.3–28.3)	25.3 (23.2–27.9)	24.9 (22.7–27.4)
MET score	56.5 (37.0–84.8)	51.0 (32.3–78.0)	55.5 (36.3–84.0)	57.4 (38.0–85.0)	58.5 (38.5–87.0)	60.0 (39.8–88.5)
Smoking status						
Never	19 629 (35.4)	2737 (24.7)	3743 (33.8)	3982 (35.9)	4442 (40.1)	4725 (42.6)
Former	15 862 (28.6)	2659 (24.0)	2969 (26.8)	3203 (28.9)	3527 (31.8)	3504 (31.6)
Current	19 922 (36.0)	5692 (51.3)	4364 (39.4)	3895 (35.2)	3112 (28.1)	2859 (25.8)
Education						
⩽7 years	18 143 (32.7)	5051 (45.6)	4191 (37.8)	3539 (31.9)	2972 (26.8)	2390 (21.6)
8–10 years	25 558 (46.1)	4847 (43.7)	5211 (47.0)	5298 (47.8)	5250 (47.4)	4952 (44.7)
⩾11 years	11 684 (21.1)	1184 (10.7)	1670 (15.1)	2239 (20.2)	2852 (25.7)	3739 (33.7)
Mean household income (DKK per year)						
⩽394 700	13 634 (24.6)	3270 (29.5)	2694 (24.3)	2658 (24.0)	2535 (22.9)	2477 (22.3)
394 701–570 930	13 842 (25.0)	3238 (29.2)	2959 (26.7)	2683 (24.2)	2565 (23.1)	2397 (21.6)
570 931–758 297	13 953 (25.2)	2909 (26.2)	3011 (27.2)	2870 (25.9)	2598 (23.4)	2565 (23.1)
> 758 297	13 984 (25.2)	1671 (15.1)	2412 (21.8)	2869 (25.9)	3383 (30.5)	3649 (32.9)
Hypertensive	9288 (16.8)	1839 (16.6)	1891 (17.1)	1888 (17.0)	1846 (16.7)	1824 (16.5)
Hypercholesterolemic	4138 (7.5)	902 (8.1)	820 (7.4)	845 (7.6)	848 (7.7)	723 (6.5)
Comorbidities						
Diabetes	1158 (2.1)	275 (2.5)	215 (1.9)	249 (2.2)	211 (1.9)	208 (1.9)
Ischaemic heart disease	2116 (3.8)	561 (5.1)	403 (3.6)	424 (3.8)	383 (3.5)	345 (3.1)
Ischaemic stroke	769 (1.4)	214 (1.9)	145 (1.3)	145 (1.3)	130 (1.2)	135 (1.2)
CKD	200 (0.4)	42 (0.4)	33 (0.3)	43 (0.4)	42 (0.4)	40 (0.4)
Medication use						
Insulin treated	683 (1.2)	158 (1.4)	121 (1.1)	152 (1.4)	129 (1.2)	123 (1.1)
Antihypertensive	6797 (12.3)	1337 (12.1)	1398 (12.6)	1379 (12.4)	1348 (12.2)	1335 (12.0)
Statin	1085 (2.0)	265 (2.4)	214 (1.9)	222 (2.0)	213 (1.9)	171 (1.5)
HRT						
Never	15 810 (54.4)	2584 (55.2)	3014 (55.7)	3241 (55.9)	3233 (52.5)	3738 (53.3)
Current	8742 (30.1)	1282 (27.4)	1551 (28.7)	1682 (29.0)	1996 (32.4)	2231 (31.8)
Former	4478 (15.4)	803 (17.2)	838 (15.5)	871 (15.0)	923 (15.0)	1043 (14.9)
NSAID	17 934 (32.6)	3493 (31.7)	3493 (31.8)	3594 (32.6)	3589 (32.5)	3765 (34.2)
Aspirin	6983 (12.6)	1362 (12.3)	1345 (12.1)	1420 (12.8)	1370 (12.4)	1486 (13.4)
Dietary characteristics						
Energy (kcal)	2271 (1878–2717)	2060 (1680–2484)	2214 (1844–2629)	2330 (1944–2768)	2375 (1988–2824)	2373 (1976–2842)
Total fish intake (g per day)	38 (25–55)	33 (22–49)	38 (25–54)	40 (27–57)	41 (28–59)	40 (27–57)
Red meat intake (g per day)	78 (56–107)	80 (58–108)	81 (59–110)	80 (58–110)	78 (57–107)	72 (52–99)
Processed meat intake (g per day)	25 (14–40)	28 (17–45)	26 (15–42)	25 (14–40)	23 (14–38)	20 (11–34)
Refined grain intake (g per day)	46 (29–72)	45 (27–80)	46 (29–73)	47 (30–72)	46 (30–70)	45 (30–68)
Wholegrain intake (g per day)	128 (86–175)	116 (72–165)	123 (84–171)	126 (86–173)	135 (97–181)	144 (103–193)
Dietary fibre intake (g per day)	20 (16–25)	16 (13–20)	19 (16–23)	21 (17–25)	22 (18–27)	23 (19–29)
Saturated FA (g per day)	31 (24–39)	29 (23–37)	31 (24–39)	32 (24–40)	32 (25–41)	32 (24–41)
Polyunsaturated FA (g per day)	13 (10–17)	12 (9–16)	13 (10–17)	14 (10–18)	14 (11–18)	14 (10–18)
Monounsaturated FA (g per day)	27 (21–35)	26 (20–34)	27 (21–35)	28 (22–35)	28 (22–35)	27 (21–34)
Fruit intake (g per day)	171 (95–281)	87 (44–141)	161 (98–238)	193 (114–301)	224 (140–360)	240 (141–390)
Vegetable intake (g per day)	162 (105–231)	114 (71–170)	150 (100–212)	168 (114–235)	185 (127–254)	196 (135–272)
Alcohol intake (g per day)	13 (6–31)	11 (3–23)	13 (6–25)	15 (6–34)	14 (7–32)	13 (6–32)

Data expressed as median (IQR) or n (%), unless otherwise stated. Q: quintile; BMI: body mass index; MET: metabolic equivalent; DKK: Danish Krone; CKD: chronic kidney disease; HRT: hormone replacement therapy; NSAID: nonsteroidal anti-inflammatory drug; FA: fatty acids.

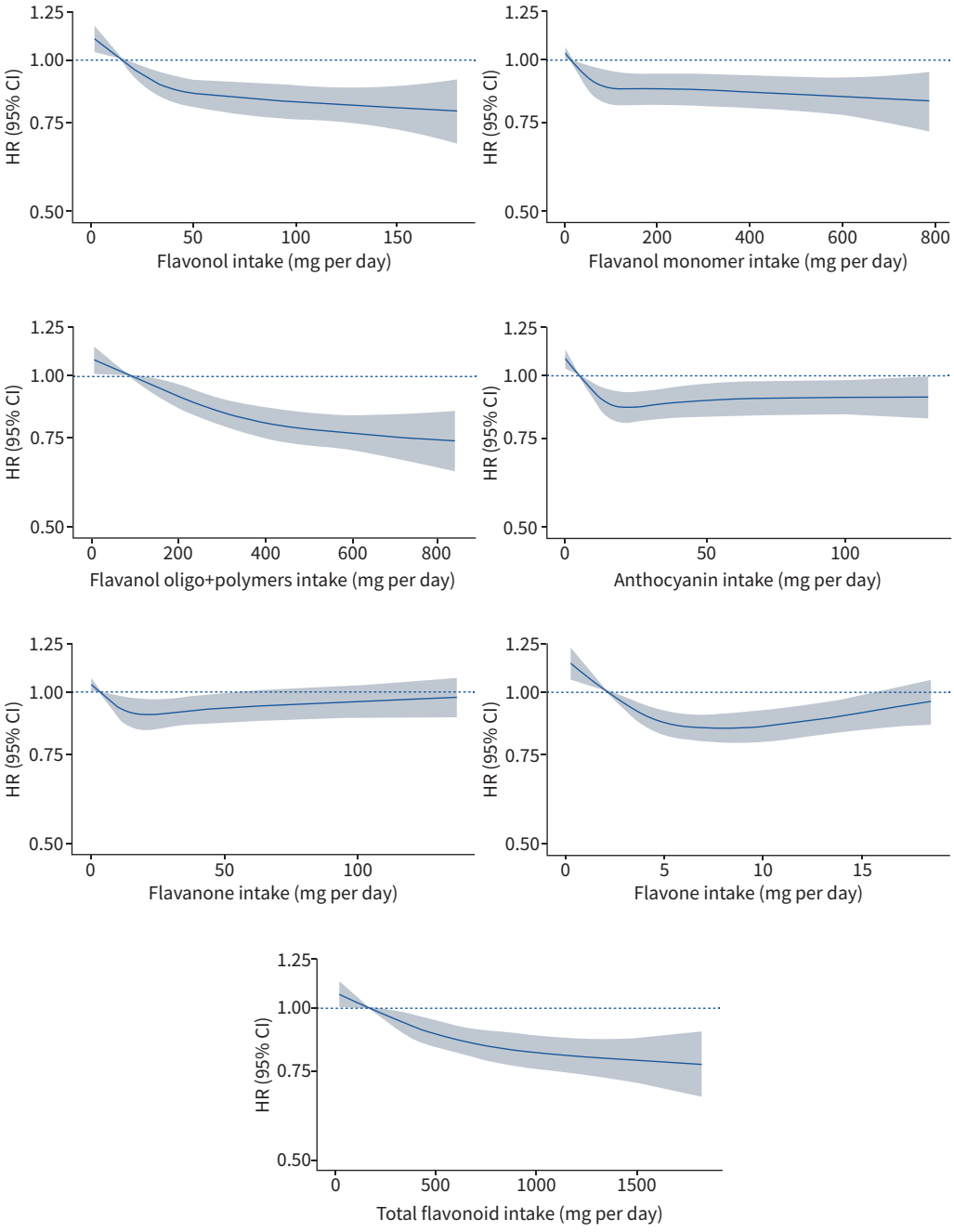

FIGURE 1 Cubic spline curves describing the association between total flavonoid and flavonoid subclass intakes and chronic obstructive pulmonary disease (COPD)-related healthcare visits in participants of the Danish Diet, Cancer and Health cohort (n=55 413). Hazard ratios and 95% confidence intervals are based on Cox proportional hazards models adjusted for age, sex, body mass index, smoking status, smoking pack-years, physical activity, education, social economic status (income) and alcohol intake (Model 1b) and compare the specific level of flavonoid intake (horizontal axis) to the median intake for participants in the lowest intake quintile.

present study, the "average" cohort participant who was both a current smoker and a low flavonoid consumer at baseline had between an ~6% (women) and ~5% (men) higher risk of COPD than their high-flavonoid-consuming counterparts. Furthermore, a high flavonoid intake appeared to lessen, but not negate, the risk of COPD associated with higher smoking intensity.

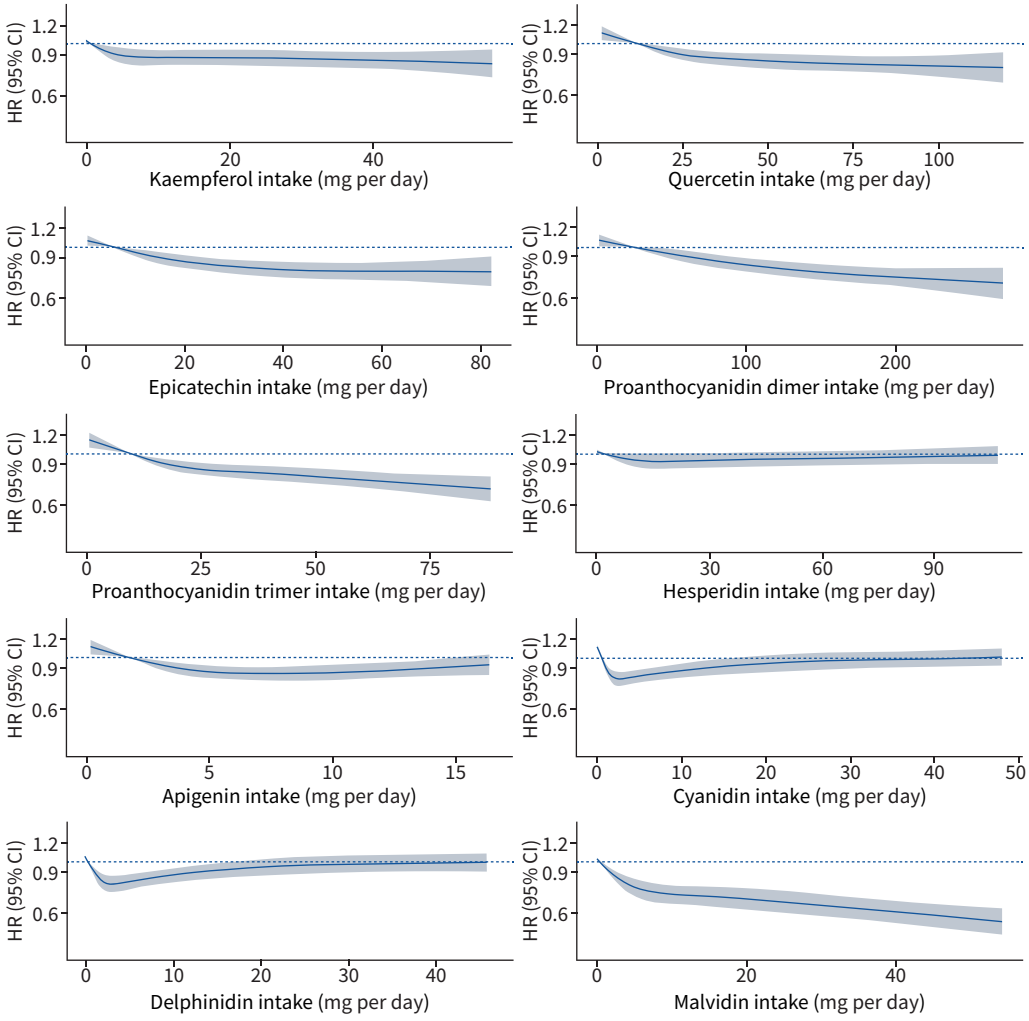

FIGURE 2 Cubic spline curves describing the association between major flavonoid compound intakes and chronic obstructive pulmonary disease (COPD)-related healthcare visits in participants of the Danish Diet, Cancer and Health cohort (n=55 413). Hazard ratios and 95% confidence intervals are based on Cox proportional hazards models adjusted for age, sex, body mass index, smoking status, smoking pack-years, physical activity, social economic status (income) and alcohol intake (Model 1b) and are comparing the specific level of flavonoid intake (horizontal axis) to the median intake for participants in the lowest intake quintile.

In 2001, the first study to examine the association between dietary flavonoid intake and lung function reported a beneficial inverse association between intakes of catechins, flavonols and flavones (the only flavonoid subclasses for which food content data were available at the time) and forced expiratory volume in 1 s (FEV_1) [26]. Despite these findings, there have been very few studies since. A longitudinal analysis of participants of the Veterans Affairs Normative Aging Study [14] demonstrated inverse associations between anthocyanin intake and age-related decline in lung function, a risk factor for COPD [27], in current, former and never-smokers. More recently, two cross-sectional studies reported protective associations between various flavonoid subclasses and pulmonary function parameters [12, 13]. To our knowledge, the present study is the first to investigate associations between dietary flavonoid intakes and incident COPD. Our findings of clear inverse associations for all flavonoid subclasses, after multivariable adjustments for demographic, lifestyle and dietary confounders, highlights the need for further research in this important, yet sparsely investigated, area. Furthermore, that we see evidence of a plateau in the association, even in high-risk groups, points to optimal flavonoid intakes that are achievable with diet alone.

In the present study, the association between flavonoid intake and COPD was only present in current and former smokers and the absolute risk of COPD in both current and former smokers was lower for those with the highest flavonoids intakes; this aligns with the hypothesis that flavonoids may work to counteract,

TABLE 2 Hazard ratios for chronic obstructive pulmonary disease by quintiles of flavonoid intake

	Flavonoid intake quintiles				
	Q1	Q2	Q3	Q4	Q5
Subjects, n	11 083	11 083	11 082	11 083	11 082
Total flavonoids					
Events, n	1621	1222	1085	861	768
Intake (mg per day)[#]	174 (6–251)	321 (251–395)	496 (395–602)	727 (602–910)	1203 (910–3552)
HR (95% CI)					
Model 1	ref.	0.71 (0.68–0.75)	0.55 (0.52–0.59)	0.48 (0.45–0.51)	0.43 (0.40–0.46)
Model 1b	ref.	0.94 (0.90–0.99)	0.89 (0.84–0.95)	0.85 (0.79–0.91)	0.80 (0.74–0.87)
Model 2	ref.	0.96 (0.91–1.01)	0.92 (0.86–0.98)	0.88 (0.82–0.95)	0.85 (0.78–0.92)
Flavonols					
Events (n)	1665	1286	989	869	748
Intake (mg per day)[#]	15 (0–21)	26 (21–32)	39 (32–50)	66 (50–83)	116 (83–251)
HR (95% CI)					
Model 1	ref.	0.73 (0.70–0.76)	0.57 (0.53–0.60)	0.45 (0.42–0.49)	0.42 (0.39–0.45)
Model 1b	ref.	0.93 (0.89–0.97)	0.88 (0.83–0.94)	0.85 (0.79–0.91)	0.82 (0.76–0.89)
Model 2	ref.	0.95 (0.91–1.00)	0.92 (0.86–0.98)	0.89 (0.83–0.96)	0.88 (0.81–0.95)
Flavanol monomers					
Events, n	1636	1217	1086	861	757
Intake (mg per day)[#]	14 (0–21)	30 (21–46)	67 (46–115)	261 (115–282)	474 (282–916)
HR (95% CI)					
Model 1	ref.	0.81 (0.79–0.84)	0.59 (0.55–0.63)	0.45 (0.42–0.49)	0.45 (0.41–0.48)
Model 1b	ref.	0.96 (0.93–0.99)	0.90 (0.84–0.97)	0.87 (0.81–0.94)	0.86 (0.80–0.93)
Model 2	ref.	0.97 (0.94–1.00)	0.94 (0.87–1.01)	0.92 (0.85–0.99)	0.91 (0.84–0.98)
Flavanol oligo+polymers					
Events, n	1642	1230	1016	898	771
Intake (mg per day)[#]	92 (1–136)	179 (136–217)	256 (217–303)	360 (303–434)	537 (434–2254)
HR (95% CI)					
Model 1	ref.	0.67 (0.64–0.70)	0.54 (0.51–0.57)	0.48 (0.45–0.51)	0.44 (0.41–0.47)
Model 1b	ref.	0.93 (0.88–0.98)	0.87 (0.83–0.93)	0.82 (0.77–0.88)	0.78 (0.72–0.84)
Model 2	ref.	0.94 (0.90–0.99)	0.90 (0.84–0.95)	0.85 (0.79–0.91)	0.81 (0.74–0.87)
Anthocyanins					
Events, n	1525	998	904	1035	1095
Intake (mg per day)[#]	5 (0–10)	13 (10–17)	20 (17–24)	36 (24–53)	70 (53–397)
HR (95% CI)					
Model 1	ref.	0.64 (0.61–0.67)	0.53 (0.50–0.57)	0.59 (0.55–0.63)	0.72 (0.67–0.77)
Model 1b	ref.	0.91 (0.86–0.95)	0.87 (0.81–0.93)	0.88 (0.83–0.95)	0.91 (0.84–0.98)
Model 2	ref.	0.91 (0.86–0.96)	0.88 (0.82–0.95)	0.90 (0.84–0.97)	0.93 (0.86–1.01)
Flavanones					
Events, n	1462	1075	996	1005	1019
Intake (mg per day)[#]	3 (0–6)	9 (6–13)	17 (13–26)	32 (26–49)	70 (49–564)
HR (95% CI)					
Model 1	ref.	0.78 (0.75–0.82)	0.65 (0.60–0.70)	0.64 (0.60–0.68)	0.69 (0.64–0.74)
Model 1b	ref.	0.94 (0.90–0.99)	0.91 (0.84–0.97)	0.91 (0.86–0.97)	0.94 (0.88–1.01)
Model 2	ref.	0.94 (0.90–0.99)	0.91 (0.84–0.97)	0.91 (0.85–0.97)	0.94 (0.87–1.01)
Flavones					
Events, n	1501	1179	944	922	1011
Intake (mg per day)[#]	2 (0–3)	4 (3–4)	5 (4–6)	7 (6–9)	11 (9–51)
HR (95% CI)					
Model 1	ref.	0.70 (0.67–0.74)	0.59 (0.55–0.62)	0.55 (0.52–0.59)	0.58 (0.54–0.63)
Model 1b	ref.	0.91 (0.87–0.96)	0.87 (0.82–0.92)	0.85 (0.8–0.90)	0.87 (0.81–0.93)
Model 2	ref.	0.92 (0.87–0.97)	0.88 (0.82–0.93)	0.86 (0.8–0.92)	0.88 (0.81–0.95)

Hazard ratios (95% confidence intervals) for chronic obstructive pulmonary 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 1b adjusted for age, sex, body mass index, smoking status, smoking pack-years, physical activity, alcohol intake, education and socioeconomic status (income); Model 2 adjusted for all covariates in Model 1b plus energy intake and fish, red meat, processed meat, whole grains, refined grains, polyunsaturated fatty acids, monounsaturated fatty acids and saturated fatty acids. Q: quintile. [#]: median (range).

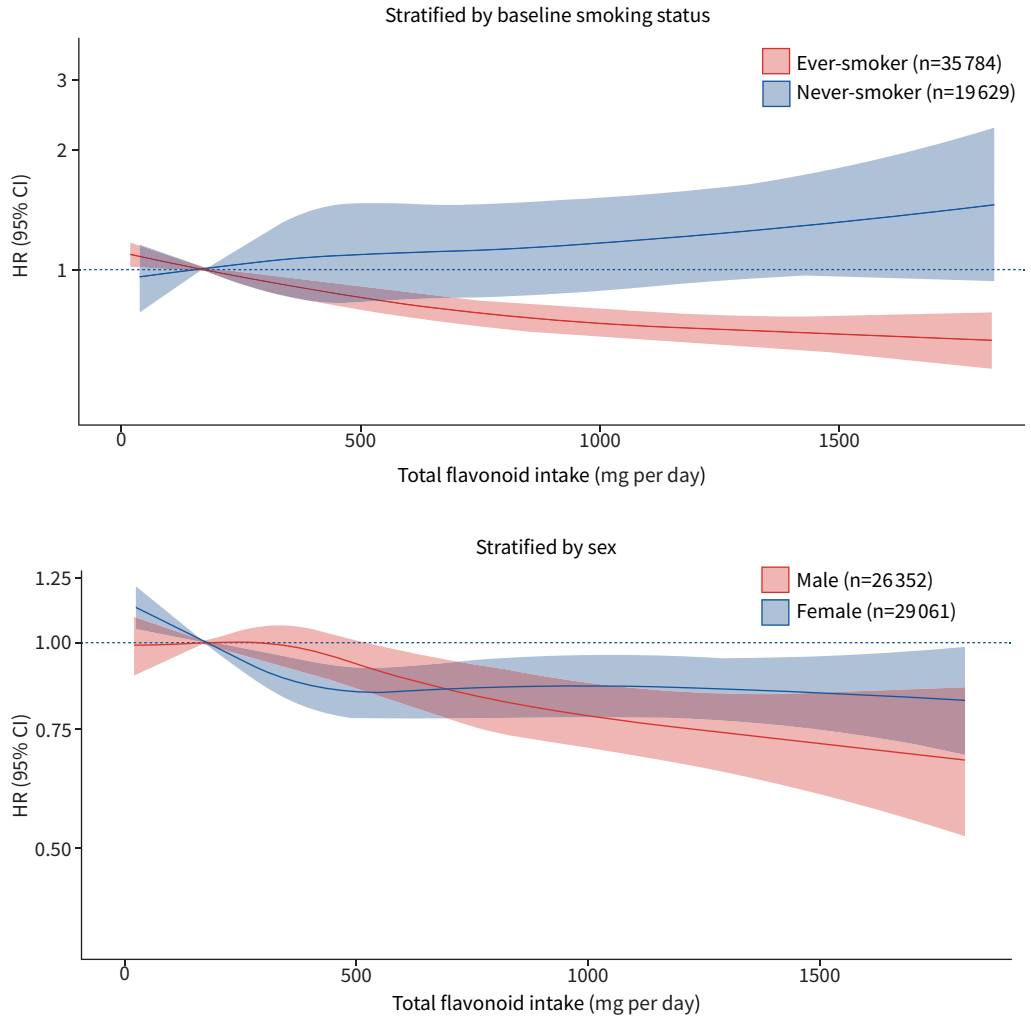

FIGURE 3 Multivariable-adjusted association between total flavonoid intake and chronic obstructive pulmonary disease (COPD)-related healthcare visits stratified by baseline smoking status and sex. Hazard ratios and 95% confidence intervals are based on Cox proportional hazards models and are comparing the specific level of flavonoid intake (horizontal axis) to the median intake for participants in the lowest intake quintile (174 mg per day). All analyses were standardised for age, sex, body mass index, smoking status, smoking pack-years, physical activity, social economic status (income), education and alcohol intake (Model 1b).

in part, the increases in systemic inflammation and oxidative stress induced by smoking [28] that give rise to COPD [29]. This hypothesis is supported by findings from our *post hoc* exploratory analysis that the risk of COPD associated with a higher number of smoking pack-years is lower in participants who consume more flavonoid-rich foods. There is a substantial overlap between pathways involved in cigarette smoke-induced inflammation and oxidative stress [29, 30] and those identified as being modulated by flavonoids [31]. That flavonoids may protect against COPD by inhibiting oxidative stress and inflammation through these signalling pathways has been demonstrated in a cigarette smoke-induced COPD mouse model supplemented with flavonoids extracted from loquat leaves [32] and a cigarette smoke-induced COPD rat model in which the animals were supplemented with the flavonoid fisetin [33]. While human intervention studies are lacking, a 2-week supplementation with flavonoid-rich grape juice decreased smoking-induced inflammation in a randomised controlled trial conducted in healthy smokers [34]. In the present study, on the relative scale, the association between total flavonoid intake and COPD was apparent in both men and women, although the shapes of the associations appeared to differ. The reasons underpinning this are difficult to determine given that, due to the complexity of interacting physiological, behavioural and/or genetic factors, it is not yet fully understood why women have a higher risk of COPD than men [35].

TABLE 3 20-year predicted risk of chronic obstructive pulmonary disease

	Total flavonoid intake		Risk difference (%)
	Q1 risk (95% CI)	Q5 risk (95% CI)	
Male			
Never-smoker	2.35 (2.07–2.66)	1.60 (1.39–1.84)	0.75
Former smoker	6.26 (5.68–6.89)	4.32 (3.85–4.84)	1.94
Current smoker	18.53 (17.31–19.82)	13.32 (12.10–14.65)	5.21
Female			
Never-smoker	3.06 (2.71–3.45)	2.09 (1.84–2.37)	0.97
Former smoker	8.06 (7.31–8.87)	5.59 (5.02–6.22)	2.47
Current smoker	22.99 (21.53–24.52)	16.79 (15.41–18.26)	6.20

The 20-year predicted risks (%) of chronic obstructive pulmonary disease calculated from logistic regression models. Unless indicated by the stratification variable, these estimates are for a smoking participant aged 56 years with a body mass index of 25.5 kg·m^{-2}, a total daily metabolic equivalent score of 56, a mean household income of 394 701–570 930 DKK per year and an alcohol intake of 13 g per day.

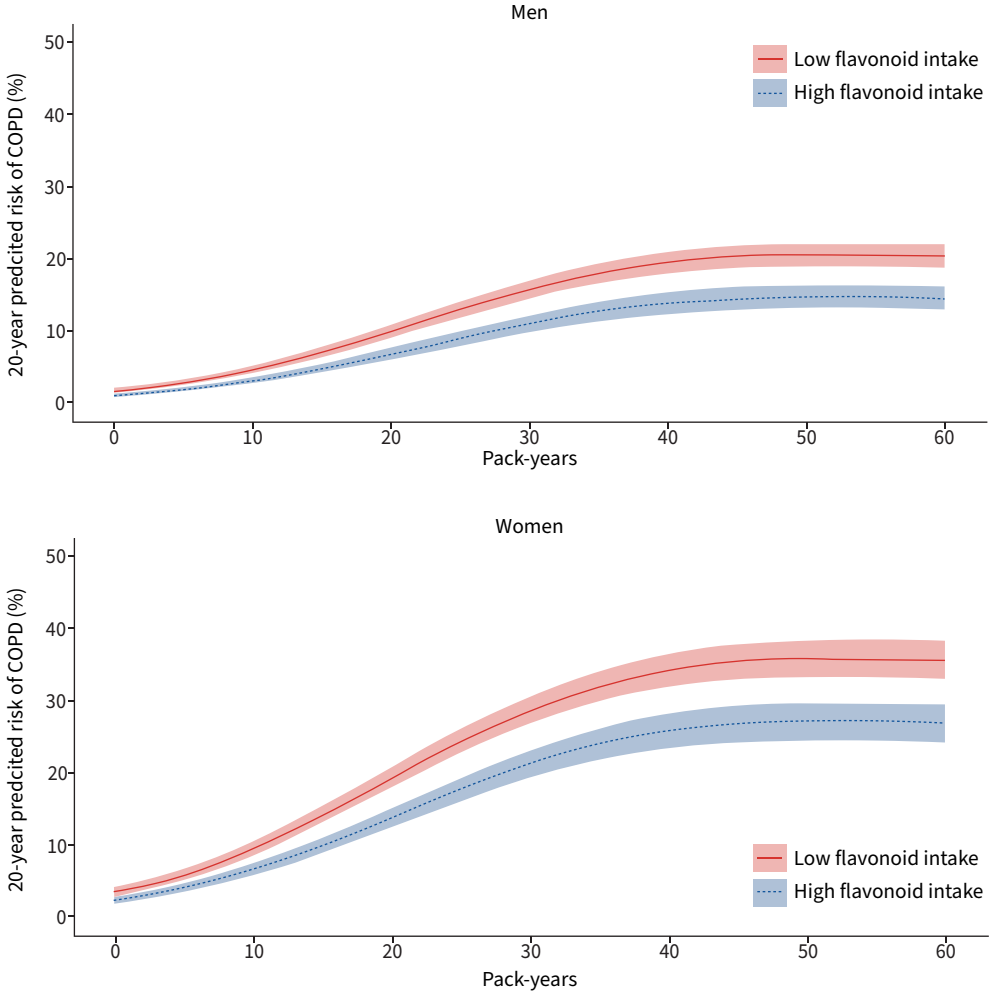

FIGURE 4 The 20-year predicted risks (%) and 95% confidence intervals of chronic obstructive pulmonary disease (COPD) by smoking pack-years for high (quintile 5) *versus* low (quintile 1) total flavonoid intakes, presented separately for men and women. The predicted risks are calculated from logistic regression models and are for a participant aged 56 years, with a body mass index of 25.5 kg·m^{-2}, a total daily metabolic equivalent score of 56, a mean household income of 394 701–570 930 DKK per year and an alcohol intake of 13 g per day.

The 20-year absolute risk of COPD for the "average" cohort participant who was both a current smoker and a low flavonoid consumer was ~6% (for women) or ~5% (for men) higher than for their high-flavonoid-consuming counterparts. This risk difference was less for former smokers (~2% each for men and women) although their risk of COPD was approximately one third that of current smokers. Reducing COPD cases by ~2% in former smokers and ~5–6% in current smokers would have a huge public health impact, which leads to the question of whether dietary modifications should be of higher priority in current and former smokers. However, irrespective of flavonoid intake, both current and former smokers had a substantially higher risk of COPD (~8- and ~3-fold higher, respectively) than non-smokers, reminding us that targeting smoking cessation is, and must continue to be, the top priority for reducing COPD risk.

The current study has numerous strengths, including a large adult population followed for 23 years with a relatively large number of events, allowing us to examine associations in subpopulations, and a negligible loss to follow-up, allowing for the estimation of absolute risks. Although our hypothesis and subsequent findings are supported by mechanistic evidence, this study is observational in nature and thus we cannot confirm causality. We also acknowledge that persons in this cohort with a higher flavonoid intake tended to have healthier diet and lifestyle and we cannot rule out the possibility of residual or unmeasured confounding. Furthermore, there may have been misclassification in the exposures because diet was self-reported using an FFQ and may have changed over time. Likewise, smoking status and intensity were only captured at baseline and thus changes during follow-up could not be accounted for. In the Danish National Patient Register, COPD diagnoses are underreported and diagnosis in primary care only would not have been captured, which may have led to lower estimation of COPD events during follow-up [21]. However, these exposure and outcome misclassifications would likely bias examined associations towards the null. Moreover, clinical data on COPD severity such as spirometry data and symptom severity of COPD were not available.

We show that current and former smokers consuming a flavonoid-rich diet have a lower risk of COPD than those with low flavonoid intakes. While the findings of this study suggest an importance of dietary flavonoids in partially mitigating the risk of COPD in people who smoke, both current and former smokers remained at a substantially higher risk of COPD than non-smokers, indicating that dietary modifications should be secondary to smoking cessation.

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

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