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Higher Habitual Flavonoid Intakes Are Associated with a Lower Incidence of Diabetes

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ABSTRACT

Background: Higher flavonoid intakes are hypothesized to confer protection against type 2 diabetes mellitus.

Objectives: We aimed to *1*) investigate associations between flavonoid intakes and diabetes, *2*) examine the mediating impact of body fat, and *3*) identify subpopulations that may receive the greatest benefit from higher flavonoid intakes in participants of the Danish Diet, Cancer, and Health Study followed up for 23 y.

Methods: Cross-sectional associations between baseline flavonoid intake, estimated using FFQs and the Phenol Explorer database, and body fat, estimated by bioelectrical impedance, were assessed using multivariable-adjusted linear regression models. Nonlinear associations between flavonoid intake and incident diabetes were examined using restricted cubic splines with multivariable-adjusted Cox proportional hazards models.

Results: Among 54,787 participants (median age: 56 y; IQR: 52–60 y; 47.3% men), 6700 individuals were diagnosed with diabetes. Participants in the highest total flavonoid intake quintile (median, 1202 mg/d) had a 1.52 kg lower body fat (95% CI: –1.74, –1.30 kg) and a 19% lower risk of diabetes (HR: 0.81; 95% CI: 0.75, 0.87) after multivariable adjustments and compared with participants in the lowest intake quintile (median: 174 mg/d). Body fat mediated 57% (95% CI: 42, 83%) of the association between flavonoid intake and incident diabetes. Of the flavonoid subclasses, moderate to high intakes of flavonols, flavanol monomers, flavanol oligo + polymers, and anthocyanins were significantly associated with a lower risk of diabetes. Although associations were not modified by sex, smoking status, BMI, or physical activity ($P_{interaction} > 0.05$ for all), findings on an absolute scale suggest that those at a higher risk (those with obesity) may benefit the most from a higher flavonoid intake.

Conclusions: The findings reported in this study suggest that a diet abundant in flavonoid-rich foods may help ameliorate diabetes risk, in part through a reduction in body fat. *J Nutr* 2021;00:1–10.

Keywords: flavonoids, diabetes, body fat, prospective cohort study, obesity

Introduction

Diabetes, a leading cause of disability worldwide (1), results in >2 million deaths every year due to the neurological and vascular complications associated with this condition (2). Strategies to lessen the global health and economic burden of diabetes should focus on diabetes prevention through the promotion of healthy behaviors and evidence-based diets at the population level (3). Higher intakes of fruits and vegetables tend to be associated with a lower incidence of type 2 diabetes mellitus (4), with particular fruits showing stronger associations than others, not attributable to their glycemic index/load (5). Flavonoids, a class of polyphenols found ubiquitously in fruits, vegetables, and other plant-derived foods and beverages, have been proposed as potential moderators of diabetes risk (6). In vitro and animal studies highlight potential mechanisms by which flavonoids and their metabolites can modify obesity and type 2 diabetes risk through inhibition of adipogenesis, insulin signaling/secretion, and the modulation of inflammatory

pathways (7–9). Based on their chemical structure, flavonoids are categorized into subclasses, with certain subclasses being common to specific foods. Structural variance between subclasses results in differences in bioavailability and biological activity (10).

In a meta-analysis of 7 prospective cohort studies, persons with the highest intakes of flavonoids had an 11% lower risk of type 2 diabetes mellitus (RR: 0.89; 95% CI: 0.82–0.96) compared with persons with the lowest intakes (11). Of the flavonoid subclasses, significant inverse associations were observed for flavonols, flavanols, and anthocyanins. Whether these inverse associations differ in subgroups at a higher risk of diabetes remains unknown.

Mitigating weight gain during middle age has a critical public health impact, with the risk of developing diabetes being up to 70% greater in those who gain $\geq$4.5 kg between the ages of 40 and 60 y (12). The reported inverse association between flavonoid intake and both body fat (13) and weight gain during 24 y of follow-up (14) may account for, at least in part, the association between flavonoid intake and incident diabetes. Because body fat may be a better determinant of diabetes development than BMI (in kg/m^2) and waist circumference (15), estimation of the mediating effect of the association between flavonoids and body fat on diabetes risk is warranted in a large observational cohort.

Therefore, the primary aim of this study was to investigate the association of total flavonoid and flavonoid subclass intakes with body fat and incident diabetes in the Danish Diet, Cancer, and Health cohort. Secondary aims were to investigate the mediating effect of body fat and to identify subpopulations that may benefit the most from higher flavonoid intakes.

Methods

Study population

Participants of the Danish Diet, Cancer, and Health study, all between the ages of 50 and 64 y, were recruited from Copenhagen and Aarhus, Denmark, between 1993 and 1997. All Danish residents are assigned a unique number, allowing cross-linking of participants to the following nationwide registries: the Civil Registration System; the Integrated Database for Labor Market Research Database; the Danish National Prescription Registry, which holds information on all filled prescriptions from Danish pharmacies since 1994, with each drug classified by the anatomical therapeutic chemical (ATC) code (16); and The Danish National Patient Register, which contains information on all hospital admissions in Denmark since 1978. This includes 1 primary diagnosis and $\geq$1 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 (17).

Of the 57,053 participants initially recruited into the study, 56,468 completed an FFQ and had no diagnosis of cancer prior to study enrolment. Participants were excluded if they had improbable energy intakes (<500 kcal/d or >5000 kcal/d; $n = 198$), missing data or extreme values for any covariates ($n = 243$), or prevalent diabetes at baseline ($n = 1240$); prevalent diabetes was defined as self-reported diabetes, ICD-10 diagnosis of diabetes (E10, E11), or use of insulin and other glucose-lowering medications (ATC; A10A, A10B) at or prior to baseline. This left 54,787 participants remaining for analysis in the current study (**Supplemental Figure 1**).

This study was approved by the Danish Data Protection Agency (Ref. 2012–58–0004 I-Suite nr: 6357, VD-2018–117).

Exposures

Exposures were baseline intakes of total flavonoids, flavonoid subclasses, individual flavonoid compounds with mean intakes >5 mg/d, and flavonoid-rich foods. Calculations of flavonoid intake are described in detail elsewhere (18). Briefly, participants completed a validated, 192-item FFQ at study entry (19); estimates of the flavonoid content of each food and beverage item in the FFQ were obtained from the Phenol-Explorer database (20). Because the average intakes of isoflavones, dihydrochalcones, dihydroflavonols, and chalcones were very low in this cohort (<5 mg/d), these flavonoid subclasses were not assessed discretely. Total flavonoid intake was calculated by summing the estimated intakes for each of the 219 flavonoid aglycones.

Study outcomes

Body fat.

Body fat (kg) at baseline was estimated using bioelectrical impedance (BIA 101-F device; Akern/RJL) as described previously (21). The method used has been validated in a Danish population, aged 35–65 y, against measurements of total body potassium and total body water (22) and has been shown to have good agreement with body composition determined by DXA in middle-aged participants from the Leiden Longevity Study (23).

Incident diabetes.

The outcome of the study consisted of either a primary or a secondary diagnosis of diabetes (ICD-10; E10, E11) for both inpatient and outpatient visits during follow-up. To capture events in people treated in a primary care setting, we included those who filled a prescription for insulin or noninsulin medication for diabetes treatment (ATC; A10A, A10B). This definition is based on that developed by the Danish Health Data Authority for identifying patients with diabetes and has a positive predictive value of 96.9% (95% CI: 89.5, 99.2%) (24).

Covariates

Participants completed questionnaires upon study enrolment, providing information on sex, age, education, smoking habits, alcohol consumption, daily activity, use of hormone replacement therapy, and diet. Anthropometry was measured at the study centers at baseline. Average annual income over 5 y was used to represent socioeconomic status and was defined as household income after taxation and interest, using the value of the Danish currency in 2015. The presence of hypertension was defined by a combination of self-reported hypertension and the use of $\geq$2 antihypertensive medications at baseline (24).

Statistical analysis

Two types of analyses were performed, a cross-sectional analysis and a time-to-event analysis. First, a cross-sectional analysis was performed to investigate the association between total flavonoid intake and body fat at baseline; a linear regression model was fit with total flavonoid intake as a continuous predictor and body fat as the response. Second, Cox proportional hazards models were used to investigate the relation between flavonoid intake and diabetes. Participants were followed from the date of enrolment until the date of diabetes diagnosis, death, emigration, or end of follow-up (August 2017), whichever came first. Quintiles were derived separately for each exposure variable. Restricted cubic splines were used to investigate nonlinear relations between

flavonoid intake (continuous) and diabetes, with HRs and 95% CIs derived from Cox proportional hazards models. HR estimates were reported for the median intake in each quintile, using the median intake in quintile 1 as the reference point. We tested for nonlinearity using a chi-square test comparing nested models. The HRs derived from the cubic splines were graphed over a fine grid of *x*-axis values; for visual simplicity, *x*-axis values were restricted to intakes within 3 SDs of the mean for each exposure. Four models of adjustment were used in all analyses: model 1a adjusted for age, sex; model 2b adjusted for age, sex, smoking status (current, former, or never), physical activity [metabolic equivalent (MET); total daily MET], pure alcohol intake (g/d), social economic status (income), hormone replacement therapy use (current, former, or never), and education (≤7 y, 8–10 y, or ≥11 y); model 2 adjusted for all covariates in model 1b plus body fat (kg), hypertension status (yes/no), and cholesterol status (yes/no); and model 3 adjusted for all covariates in model 2 plus energy intake and intakes (g/d) of red meat, processed meat, PUFAs, MUFAs, SFAs, fiber, soft drinks, coffee, and added sugar. Covariates were chosen a priori to the best of our knowledge of potential confounders of flavonoid intake and diabetes. Cox proportional hazards assumptions were tested using log–log plots of the survival function compared with time and assessed for parallel appearance. All deaths were censored rather than treated as a competing risk. To identify subpopulations that may benefit the most from higher flavonoid intakes, first, interaction on the multiplicative scale was assessed by likelihood ratio tests of Cox proportional hazards models with and without the interaction terms. Second, analyses were stratified by risk factors for diabetes, namely smoking status (never/former smoker compared with current smoker), BMI (>30 compared with <30), physical activity (greater than compared with less than the median MET score), and sex. Because there is potential for residual confounding when stratifying by smoking status, BMI, and MET score, the corresponding continuous variables (smoking pack-years, BMI, and MET score) were included in the model. In addition, due to the miniscule loss to follow-up in this cohort (<0.3%), standard logistic regression models were used to obtain the predicted 20-y absolute risk estimates of diabetes for each subgroup. For these analyses, a binary outcome indicating a diagnosis of diabetes during 20 y of follow-up was used. Unless indicated by the stratification variable, these estimates are for the "average" cohort participant at baseline—that is, a nonsmoking participant, aged 56 y, who has completed 8–10 y of education, with a BMI of 25.5, a total daily MET score of 56, a mean household income of 394,701–570,930 Danish krone/y, an alcohol intake of 13 g/d, and who is not taking hormone replacement therapy. For flavonoid exposures for which clear inverse associations with body fat and incident diabetes were observed, the extent to which the association between flavonoid intake and diabetes was mediated by baseline body fat was quantified through natural direct and indirect effects [25], which estimate how large an association we would observe if flavonoid intake had no impact on body fat (the natural direct effect) and if flavonoid intake only had an impact upon diabetes through its impact on body fat (the natural indirect effect), respectively. Estimation was done using a Cox proportional hazards model in the Medflex package for R [26]. CIs were obtained by bootstrapping (1000 iterations). Body fat was included as a continuous variable in the mediation analysis, whereas total flavonoid intake was included as a categorical variable (quintiles). In supplementary analyses, Pearson's correlations were performed between flavanone intake and the primary dietary sources of flavanones and the association between body fat (per 1.52 kg), and incident diabetes was investigated using a Cox proportional hazards model. All analyses were undertaken using Stata/IC 14.2 (StataCorp) and R statistics (R Foundation for Statistical Computing).

Results

This population of 54,787 Danish residents, with a median age of 56 y (IQR: 52–60 y) at entry, had a median follow-up of 20.8 y (IQR: 17.3–21.6 y). During 1,010,191 person-years of follow-up, 6700 individuals were diagnosed with diabetes.

Baseline characteristics

The median intake of flavonoids was 495 mg/d (IQR: 287–213 mg/d). Compared with participants with the lowest flavonoid intakes, those with the highest intakes were more likely to be women, be more physically active, have a higher degree of education, have a higher income, and less likely to have ever smoked or to be hypercholesterolemic. Furthermore, they tended to have a healthier underlying dietary pattern, consuming more fish, dietary fiber, fruits, vegetables, and tea and consuming less red meat and processed meat (**Table 1**).

Association between flavonoid intake and baseline body fat

Total flavonoid intake was inversely associated with body fat in a linear dose–response manner (*P* for trend < 0.001) across the range of intakes in this cohort. After multivariable adjustments and compared with participants in quintile 1, participants in quintiles 2–5 had a 0.39 kg (95% CI: –0.60, –0.17 kg), 0.62 kg (95% CI: –0.84, –0.41 kg), 0.90 kg (95% CI: –1.11, –0.68 kg), and 1.52 kg (95% CI: –1.74, –1.30 kg) lower body fat, respectively (model 1b; **Supplemental Table 1**). In order to aid interpretation of this, in supplementary modeling, a 1.52-kg higher body fat translated to a 12% higher risk of diabetes (HR: 1.12; 95% CI: 1.11, 1.12). For the individual flavonoid subclasses, inverse associations with baseline body fat were observed for the flavonols, flavanol monomers, flavanol oligo + polymers, and anthocyanins (Supplemental Table 1).

Association between flavonoid intake and incident diabetes

The association between total flavonoid intake and incident diabetes was nonlinear (*P* for nonlinearity = 0.013); the inverse association was steeper at lower intakes, and the gradient started to decrease at ∼400 mg/d (**Figure 1**). Compared with participants in quintile 1, participants in quintile 5 had a 19% lower risk of diabetes (HR: 0.81; 95% CI: 0.75, 0.87) after multivariable adjustments (model 1b; **Table 2**).

For flavonoid subclasses, comparing high with low intakes (quintile 5 compared with quintile 1), the risk of diabetes was 19% lower for flavonols (HR: 0.81; 95% CI: 0.75, 0.87), 16% lower for flavanol monomers (HR: 0.86; 95% CI: 0.78, 0.90), and 23% lower for flavanol oligo + polymers (HR: 0.77; 95% CI: 0.72, 0.83) after multivariable adjustments (model 1b; **Table 2**). Conversely, the risk of diabetes appeared to be positively associated with flavanone intakes, and the association with anthocyanin and flavone intakes was U-shaped (**Figure 1**). After adjusting for potential dietary confounders, flavanol oligo + polymers were significantly inversely associated with diabetes, flavanones were positively associated with diabetes, and the association between anthocyanin and flavone intake and diabetes was U-shaped (model 3; **Table 2**).

Mediation analysis

The mediation analysis showed that after multivariable adjustments (model 1b), baseline body fat explained 57% (95% CI: 42, 83%) of the total association between total flavonoid intake and incident diabetes (**Supplemental Table 2**). For individual flavonoid subclasses, for which clear inverse associations between both baseline body fat and incident diabetes were observed (i.e., flavonols, flavanol monomers, and flavanol oligo + polymers), baseline body fat explained 52% (95% CI: 35, 81%), 66% (95% CI: 45, 89%), and 35% (95% CI: 26, 48%), respectively (Supplemental Table 2).

TABLE 1 Baseline characteristics of study population[1]

	Total population (n = 54,787)	Total flavonoid intake quintiles				
		Quintile 1 (n = 10,958)	Quintile 2 (n = 10,957)	Quintile 3 (n = 10,957)	Quintile 4 (n = 10,957)	Quintile 5 (n = 10,958)
Total flavonoid intake, mg/d	495 [287–805]	174 [128–213]	321 [287–357]	495 [442–549]	727 [660–805]	1 202 [1025–1 435]
Sex, % men	25,903 (47.3)	6302 (57.5)	5592 (51.0)	5168 (47.2)	4851 (44.3)	3990 (36.4)
Age, y	56 [52–60]	56 [52–60]	56 [52–60]	56 [52–60]	56 [52–60]	55 [52–60]
BMI, kg/m²	25.5 [23.3–28.2]	26.1 [23.7–28.8]	25.8 [23.6–28.2]	25.6 [23.1–27.8]	25.3 [23.1–27.8]	24.9 [22.7–27.3]
Body fat, kg	22.3 [17.8–27.7]	22.8 [18.1–28.5]	22.7 [18.2–28.2]	22.4 [17.9–27.8]	22.1 [17.8–27.4]	21.5 [17.2–26.7]
MET score	56.5 [37.0–84.8]	51.0 [32.3–78.0]	55.5 [36.3–84.0]	57.5 [38.5–85.0]	58.5 [38.8–87.0]	60.0 [40.0–88.5]
Smoking status						
Never	19,281 (35.2)	2669 (24.4)	3662 (33.4)	3928 (35.8)	4370 (39.9)	4652 (42.5)
Former	15,746 (28.7)	2630 (24.0)	2952 (26.9)	3172 (28.9)	3508 (32.0)	3484 (31.8)
Current	19,760 (36.1)	5659 (51.6)	4343 (39.6)	3857 (35.2)	3079 (28.1)	2822 (25.8)
Education, y						
≤7	17,939 (32.7)	4995 (45.6)	4156 (37.9)	3495 (31.9)	2945 (26.9)	2348 (21.4)
8–10	25,286 (46.2)	4791 (43.7)	5161 (47.1)	5253 (47.9)	5188 (47.3)	4893 (44.7)
≥11	11,537 (21.1)	1166 (10.6)	1637 (14.9)	2206 (20.1)	2818 (25.7)	3710 (33.9)
Mean household income, DKK/y						
<394,700	13,473 (24.6)	3246 (29.6)	2663 (24.3)	2625 (24.0)	2505 (22.9)	2434 (22.2)
394,701–570,930	13,659 (24.9)	3184 (29.1)	2929 (26.7)	2656 (24.2)	2533 (23.1)	2357 (21.5)
570,931–758,297	13,794 (25.2)	2876 (26.2)	2976 (27.2)	2836 (25.9)	2570 (23.5)	2536 (23.1)
>758,297	13,861 (25.3)	1652 (15.1)	2389 (21.8)	2840 (25.9)	3349 (30.6)	3631 (33.1)
Hypertensive	8901 (16.2)	1758 (16.0)	1817 (16.6)	1811 (16.5)	1768 (16.1)	1747 (15.9)
Hypercholesterolemic	3964 (7.2)	859 (7.8)	794 (7.2)	801 (7.3)	814 (7.4)	696 (6.4)
Medication use						
Antihypertensive	6480 (11.8)	1275 (11.6)	1341 (12.2)	1315 (12.0)	1280 (11.7)	1269 (11.6)
Statin	1026 (17.0)	252 (18.2)	208 (16.6)	206 (17.3)	197 (17.3)	163 (15.2)
HRT (women only)						
Never	15,701 (54.4)	2568 (55.2)	2988 (55.7)	3231 (55.8)	3206 (52.5)	3708 (53.2)
Current	8707 (30.1)	1278 (27.4)	1550 (28.9)	1677 (29.0)	1983 (32.5)	2219 (31.8)
Former	4476 (15.5)	810 (17.4)	827 (15.4)	881 (15.2)	917 (15.0)	1041 (14.9)
Dietary characteristics						
Energy, kcal/d	2270 [1877–2716]	2059 [1679–2484]	2213 [1844–2629]	2327 [1941–2766]	2375 [1989–2825]	2372 [1976–2837]
Total fish intake, g/d	38 [25–55]	33 [22–48]	37 [25–54]	40 [27–57]	41 [28–59]	40 [27–57]
Red meat intake, g/d	78 [56–107]	80 [58–108]	81 [59–109]	80 [58–110]	78 [57–107]	72 [52–99]
Processed meat intake, g/d	24 [14–40]	28 [17–45]	26 [15–42]	25 [14–40]	23 [14–38]	20 [11–34]
Dietary fiber intake, g/d	20 [16–25]	16 [13–20]	19 [16–23]	21 [17–25]	22 [18–27]	23 [19–29]
SFA, g/d	31 [24–39]	29 [23–38]	31 [24–39]	32 [24–40]	32 [25–41]	32 [24–41]
PUFA, g/d	13 [10–17]	12 [9–16]	13 [10–17]	14 [10–18]	14 [11–18]	14 [10–18]
MUFA, g/d	27 [21–35]	26 [20–34]	27 [21–35]	28 [22–35]	28 [22–35]	27 [21–34]
Fruit intake, g/d	171 [94–281]	87 [44–141]	161 [97–237]	193 [114–300]	224 [140–360]	241 [141–390]
Vegetable intake, g/d	161 [104–230]	113 [71–168]	149 [99–210]	167 [113–234]	184 [126–252]	195 [135–271]
Alcohol intake, g/d	13 [6–31]	11 [4–24]	13 [6–25]	15 [6–34]	14 [7–32]	13 [6–32]
Tea intake, g/d	7 [3–500]	3 [0–16]	16 [3–86]	86 [7–200]	500 [86–500]	900 [500–1300]

[1]Values are medians [IQRs] or n (%), unless otherwise stated. CKD, chronic kidney disease; COPD, common obstructive pulmonary disease; DKK, Danish krone; HRT, hormone replacement therapy; MET, metabolic equivalent.
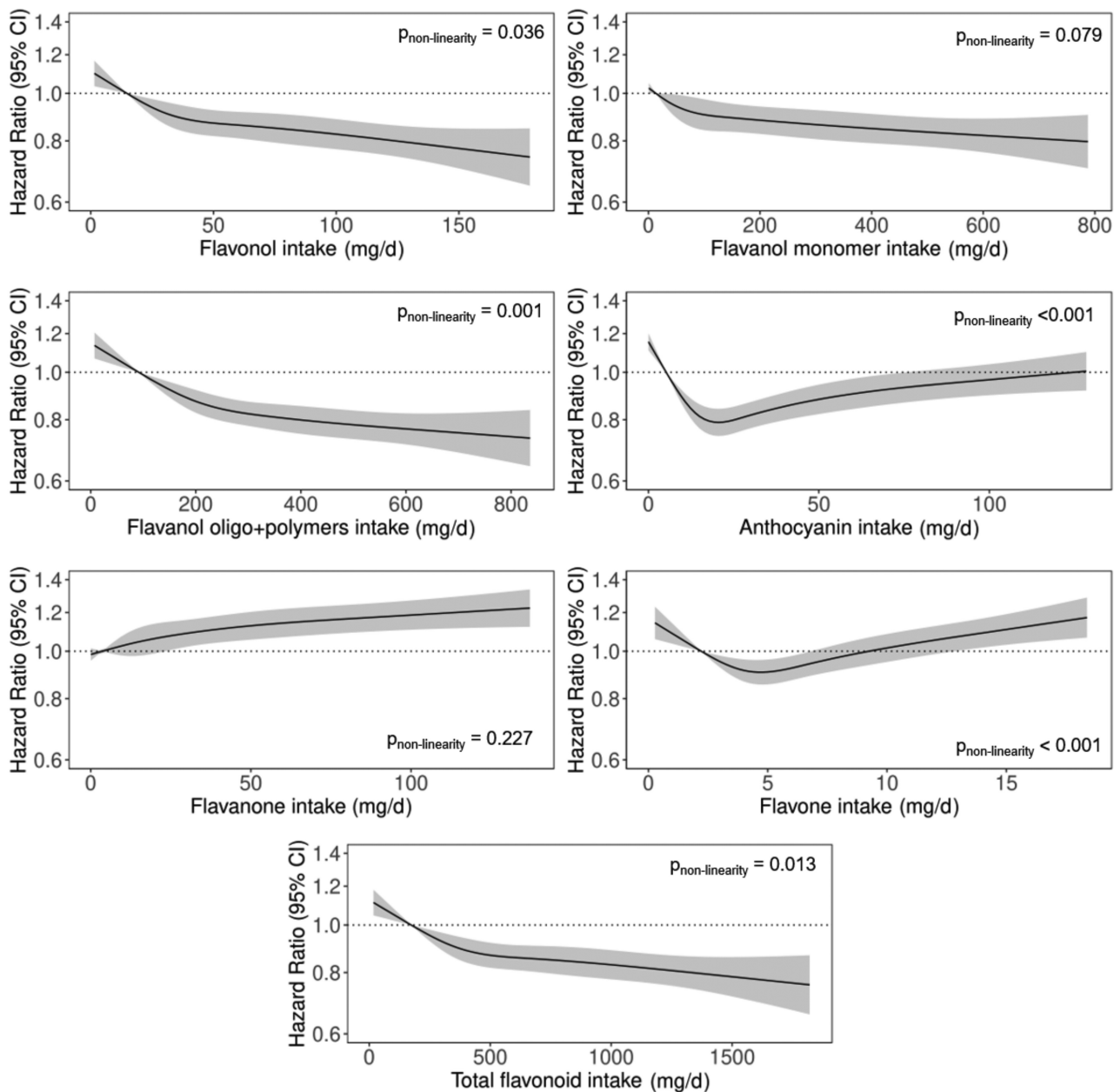
FIGURE 1 HRs based on cubic splines to describe the association between flavonoid subclass intakes (mg/d) and incident diabetes among participants of the Danish Diet, Cancer, and Health cohort ($n = 54,787$). HRs (y axis on logarithmic scale) are based on Cox proportional hazards models adjusted for age, sex, smoking status, physical activity, alcohol intake, education, hormone replacement therapy, and socioeconomic status (income) and are comparing the specific level of flavonoid intake (horizontal axis) to the median intake for participants in the lowest intake quintile. P values for nonlinearity were obtained using a chi-square test to compare nested models.

Associations between major flavonoid compound intakes and incident diabetes

Inverse associations between all individual flavonoid compounds with mean intakes >5 mg/d [except for the flavanol monomers, for which we present only the results for epicatechin due to the very high correlation (>0.89) between flavonol monomers] and incident diabetes are presented in **Supplemental Table 3** and **Supplemental Figure 2**. Kaempferol, quercetin, epicatechin, procyanidin dimer, procyanidin trimer, and malvidin intakes were significantly inversely associated with incident diabetes, although for the latter 2 associations plateaued at moderate intakes. Conversely, hesperidin intakes were positively associated with diabetes, and associations between diabetes and

apigenin, delphinidin, and cyanidin intakes were somewhat U-shaped (Supplemental Figure 2).

Associations between total flavonoid intake and incident diabetes stratified by risk factors for diabetes

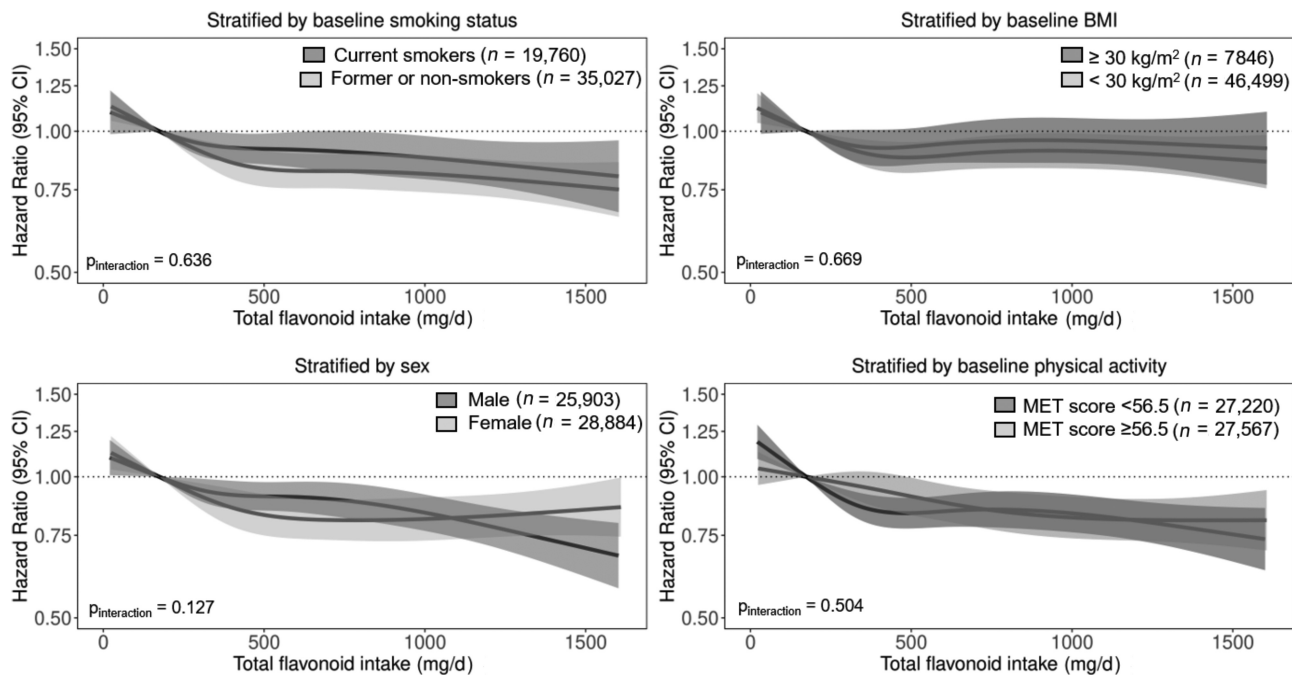The association between total flavonoid intake and incident diabetes was present in all subgroups investigated ($P_{interaction} > 0.05$ for all; **Figure 2**). However, because the prevalence of diabetes was higher in those with a BMI ≥30, the difference (total flavonoid intake quintile 5 – total flavonoid intake quintile 1) in the 20-y estimated risk of diabetes was greater in this subgroup (**Table 3**).

TABLE 2 HRs of incident diabetes by quintiles of flavonoid intake[1]

	Flavonoid intake quintiles				
	Quintile 1 (*n* = 10,958)	Quintile 2 (*n* = 10,957)	Quintile 3 (*n* = 10,957)	Quintile 4 (*n* = 10,957)	Quintile 5 (*n* = 10,958)
Total flavonoids					
No. events	1734	1380	1337	1205	1044
Intake,[2] mg/d	174 (6–251)	321 (251–395)	495 (395–602)	727 (602–909)	1202 (909–3552)
HR (95% CI)					
Model 1a	Ref	0.83 (0.80, 0.87)	0.74 (0.69, 0.78)	0.69 (0.65, 0.73)	0.63 (0.59, 0.68)
Model 1b	Ref	0.92 (0.88, 0.96)	0.87 (0.82, 0.92)	0.85 (0.80, 0.90)	0.81 (0.75, 0.87)
Model 2	Ref	0.92 (0.88, 0.96)	0.89 (0.84, 0.94)	0.89 (0.84, 0.95)	0.88 (0.82, 0.94)
Model 3	Ref	0.92 (0.88, 0.96)	0.88 (0.83, 0.94)	0.88 (0.82, 0.94)	0.84 (0.78, 0.92)
Flavonols					
No. events	1684	1510	1263	1212	1031
Intake,[2] mg/d	15 (0–20)	26 (20–32)	38 (32–50)	66 (50–83)	116 (83–251)
HR (95% CI)					
Model 1a	Ref	0.84 (0.81, 0.88)	0.75 (0.70, 0.79)	0.68 (0.64, 0.72)	0.62 (0.58, 0.66)
Model 1b	Ref	0.93 (0.89, 0.97)	0.89 (0.83, 0.94)	0.86 (0.80, 0.91)	0.81 (0.75, 0.87)
Model 2	Ref	0.91 (0.88, 0.95)	0.87 (0.82, 0.93)	0.89 (0.84, 0.95)	0.87 (0.81, 0.94)
Model 3	Ref	0.92 (0.88, 0.97)	0.88 (0.82, 0.94)	0.88 (0.82, 0.95)	0.84 (0.77, 0.91)
Flavanol monomers					
No. events	1676	1419	1369	1191	1045
Intake,[2] mg/d	14 (0–21)	30 (21–45)	66 (45–115)	261 (115–281)	473 (281–916)
HR (95% CI)					
Model 1a	Ref	0.91 (0.89, 0.94)	0.79 (0.74, 0.84)	0.70 (0.65, 0.74)	0.66 (0.62, 0.71)
Model 1b	Ref	0.97 (0.94, 1.00)	0.92 (0.86, 0.99)	0.87 (0.81, 0.93)	0.84 (0.78, 0.90)
Model 2	Ref	0.96 (0.93, 0.99)	0.91 (0.85, 0.97)	0.94 (0.88, 1.01)	0.92 (0.86, 0.99)
Model 3	Ref	1.00 (0.97, 1.03)	0.99 (0.92, 1.06)	0.95 (0.89, 1.02)	0.93 (0.87, 1.00)
Flavanol oligo + polymers					
No. events	1750	1391	1328	1189	1042
Intake,[2] mg/d	92 (0–136)	179 (136–217)	255 (217–303)	359 (303–434)	537 (434–2254)
HR (95% CI)					
Model 1a	Ref	0.80 (0.77, 0.84)	0.71 (0.68, 0.75)	0.66 (0.62, 0.70)	0.62 (0.58, 0.66)
Model 1b	Ref	0.89 (0.85, 0.94)	0.84 (0.79, 0.89)	0.81 (0.76, 0.86)	0.77 (0.72, 0.83)
Model 2	Ref	0.91 (0.87, 0.95)	0.86 (0.82, 0.91)	0.84 (0.79, 0.89)	0.82 (0.77, 0.88)
Model 3	Ref	0.93 (0.88, 0.98)	0.90 (0.85, 0.95)	0.89 (0.83, 0.95)	0.90 (0.83, 0.97)
Anthocyanins					
No. events	1632	1298	1171	1220	1379
Intake,[2] mg/d	5 (0–10)	13 (10–17)	20 (17–24)	36 (24–53)	70 (53–397)
HR (95% CI)					
Model 1a	Ref	0.76 (0.72, 0.79)	0.67 (0.63, 0.72)	0.70 (0.66, 0.74)	0.78 (0.73, 0.83)
Model 1b	Ref	0.85 (0.81, 0.89)	0.80 (0.75, 0.85)	0.84 (0.79, 0.89)	0.92 (0.85, 0.99)
Model 2	Ref	0.88 (0.84, 0.92)	0.84 (0.79, 0.90)	0.88 (0.83, 0.94)	0.94 (0.87, 1.01)
Model 3	Ref	0.87 (0.83, 0.91)	0.83 (0.78, 0.89)	0.88 (0.83, 0.94)	0.97 (0.90, 1.04)
Flavanones					
No. events	1372	1239	1356	1348	1385
Intake,[2] mg/d	4 (0–6)	9 (6–13)	18 (13–26)	32 (26–49)	70 (49–564)
HR (95% CI)					
Model 1a	Ref	0.96 (0.92, 1.01)	0.94 (0.88, 1.01)	0.96 (0.90, 1.02)	1.02 (0.95, 1.09)
Model 1b	Ref	1.03 (0.98, 1.07)	1.06 (0.99, 1.13)	1.10 (1.03, 1.17)	1.15 (1.08, 1.23)
Model 2	Ref	1.00 (0.96, 1.05)	1.02 (0.95, 1.09)	1.05 (0.99, 1.12)	1.11 (1.04, 1.19)
Model 3	Ref	1.04 (0.99, 1.08)	1.07 (1.00, 1.15)	1.10 (1.03, 1.18)	1.13 (1.05, 1.22)
Flavones					
No. events	1446	1278	1299	1271	1406
Intake,[2] mg/d	2 (0–3)	4 (3–5)	5 (5–6)	7 (6–9)	11 (9–51)
HR (95% CI)					
Model 1a	Ref	0.85 (0.81, 0.89)	0.81 (0.76, 0.86)	0.84 (0.79, 0.89)	0.92 (0.86, 0.98)
Model 1b	Ref	0.92 (0.88, 0.97)	0.91 (0.86, 0.97)	0.96 (0.90, 1.02)	1.04 (0.98, 1.12)
Model 2	Ref	0.93 (0.88, 0.97)	0.91 (0.86, 0.97)	0.95 (0.89, 1.01)	1.01 (0.95, 1.08)
Model 3	Ref	0.95 (0.90, 1.00)	0.96 (0.90, 1.02)	1.02 (0.95, 1.09)	1.12 (1.04, 1.21)

[1]HRs (95% CI) for incident diabetes during 23 y of follow-up, obtained from restricted cubic splines based on Cox proportional hazards models. Model 1a adjusted for age and sex; model 1b adjusted for age, sex, smoking status, physical activity, alcohol intake, education, hormone replacement therapy, and socioeconomic status (income); model 2 adjusted for all covariates in model 1b plus body fat, hypertension status, and cholesterol status; and model 3 adjusted for all covariates in model 2 plus energy intake and intakes (g/d) of red meat, processed meat, PUFAs, MUFAs, SFAs, fiber, soft drinks, coffee, and added sugar.
[2]Median; range in parentheses (all such values).

FIGURE 2 Multivariable-adjusted association between total flavonoid intake and incident diabetes stratified by baseline smoking status, BMI, sex, and physical activity. HRs (*y* axis on logarithmic scale) are based on Cox proportional hazard 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/d). All analyses were standardized for age, sex, smoking status, physical activity, alcohol intake, education, hormone replacement therapy, and socioeconomic status (income). MET, metabolic equivalent.

Investigation into the positive association between flavanone intake and diabetes

In this cohort, flavanone intake was most strongly correlated with intakes of oranges ($r = 0.35$), orange juice ($r = 0.89$), grapefruit ($r = 0.12$), and soft drinks ($r = 0.38$; an assumption was made that lemonade consists of ~6% lemon juice). Intakes of oranges, orange juice, and grapefruit were not significantly associated with incident diabetes, whereas intakes of soft drinks were significantly positively associated with incident diabetes (**Supplemental Figure 3**). There was no association between

TABLE 3 20-y predicted risk of diabetes[1]

	Total flavonoid intake		
	Quintile 1 risk (95% CI)	Quintile 5 risk (95% CI)	Risk difference (%)
Men			
Nonsmoker	10.55 (9.64, 11.53)	9.24 (8.36, 10.20)	1.31
Former smoker	11.84 (10.87, 12.89)	10.39 (9.44, 11.42)	1.45
Current smoker	15.03 (13.93, 16.20)	13.25 (12.07, 14.52)	1.78
BMI, kg/m^2			
<30	11.17 (10.23, 12.19)	9.38 (8.51, 10.34)	1.79
≥30	35.24 (32.91, 37.63)	30.94 (28.56, 33.43)	4.30
MET score			
<56.5	10.72 (9.79, 11.72)	9.39 (8.48, 10.37)	1.33
≥56.5	10.30 (9.40, 11.27)	9.02 (8.16, 9.96)	1.28
Women			
Nonsmoker	7.36 (6.70, 8.08)	6.42 (5.82, 7.07)	0.94
Former smoker	8.30 (7.53, 9.14)	7.24 (6.55, 8.00)	1.06
Current smoker	10.65 (9.77, 11.60)	9.33 (8.47, 10.26)	1.32
BMI, kg/m^2			
<30	7.53 (6.86, 8.25)	6.28 (5.71, 6.91)	1.25
≥30	26.04 (24.10, 28.08)	22.47 (20.66, 24.40)	3.57
MET score			
<56.5	7.48 (6.80, 8.23)	6.52 (5.91, 7.20)	0.96
≥56.5	7.18 (6.53, 7.90)	6.26 (5.68, 6.90)	0.92

[1]The 20-y predicted risk (percentage) of diabetes calculated from logistic regression models. Unless indicated by the stratification variable, these estimates are for a nonsmoking participant, aged 56 y, who has completed 8–10 y of education, with a BMI of 25.5, a total daily MET score of 56, with a mean household income of 394,701–570,930 DKK/y, an alcohol intake of 13 g/d, and who is not taking hormone replacement therapy. DKK, Danish krone; MET, metabolic equivalent.

Flavonoids and incident diabetes 7

flavanone intake and incident diabetes in participants who consumed soft drinks <1 per month (*n* = 25 773; **Supplemental Figure 4**).

Discussion

In this prospective cohort of 54,787 Danish men and women, a flavonoid-rich diet was inversely associated with incident diabetes; after adjusting for potential lifestyle and dietary confounders, this association plateaued at moderate flavonoid intakes (~400 to 600 mg/d). This association did not appear to be modified by smoking status, BMI, physical activity level, or sex; however, findings on an absolute scale suggest that those at a higher risk of diabetes (those with obesity) may benefit the most from a higher flavonoid consumption. Of the flavonoid subclasses, moderate to high intakes of flavonols, flavanol monomers, flavanol oligo + polymers, and anthocyanins were associated with a lower risk of diabetes. Cross-sectionally, total flavonoid intake was inversely associated with body fat, explaining approximately half of the association between total flavonoid intake and incident diabetes.

Limitations inherent to observational studies apply to the current study in that we are not able to infer causality or rule out residual confounding. Furthermore, we acknowledge common FFQ limitations, particularly that not all dietary sources of flavonoids, especially berries other than strawberries, were included in the questionnaire. Although flavonoid intake may have changed during the 23 y of follow-up, the resulting measurement error would likely result in a bias of observed associations toward the null. We acknowledge that although bioelectrical impedance has good agreement with measures of body fat, it is not the gold standard measure for estimating body fat, particularly in obese/morbidly obese and underweight individuals (27), and we only had a single measure at baseline and as such could not investigate changes in body fat or rule out residual confounding. Furthermore, the cross-sectional and mediation analyses should be interpreted with caution because temporality of the flavonoid–body fat association is not known. Unfortunately, we were unable to distinguish between type 1 and type 2 diabetes or capture diabetes treated nonpharmacologically in a primary care setting; due to the age of the cohort (between 50 and 64 y at baseline), we assume that the majority of incident cases were type 2 diabetes. Despite these limitations, the current study has many strengths, including a large sample size with 23 y of follow-up, allowing for the accumulation of a large number of incident cases of diabetes and thus affording sufficient power to examine associations in subgroups of interest; a negligible loss to follow-up, permitting the calculation of absolute risk differences; and the availability of key participant characteristics, enabling appropriate model adjustment to reduce residual confounding.

Evidence from both prospective cohort studies and randomized controlled trials points to dietary patterns high in fruits and vegetables, wholegrains, nuts, and legumes, low in both red and processed meats, sugar-sweetened beverages, and refined grains, and with a moderate alcohol intake, for the prevention and management of type 2 diabetes (28). The association between flavonoid-rich dietary patterns and incident type 2 diabetes has been investigated in several large prospective cohort studies, a meta-analysis of which reports a curvilinear relation between total flavonoid intake and incident type 2 diabetes, with a significantly lower risk at intakes ≥550 mg/d (11). In the current study, the gradient of the inverse association between total flavonoid intake and incident diabetes decreased beyond moderate intakes (quintile 3: 395–602 mg/d), indicating the existence of a threshold, beyond which higher intakes may afford little added benefit. Of the individual flavonoid subclasses, a lower risk of diabetes has been observed for anthocyanins (29, 30) [RR of 0.89 (95% CI: 0.82, 0.95) in meta-analysis (11)], flavanols (29, 31) [RR of 0.86 (95% CI: 0.78, 0.95) in meta-analysis (11)], flavonols (31, 32) [RR of 0.86 (95% CI: 0.80, 0.94) in meta-analysis (11)], flavanones (29, 33) [RR of 1.02 (95% CI: 0.94, 1.10) in meta-analysis (11)], and flavones (29) [RR of 0.96 (95% CI: 0.85, 1.08) in meta-analysis (11)]. Although the reported relative risks from the meta-analysis are similar to those described in the current study for flavonols, flavanols, and anthocyanins, we observed that higher intakes of flavanones were associated with a higher risk of incident diabetes. From our results, we conclude that this positive association was driven by the assumption that lemonade contains lemon juice (34) and is therefore a contributor to flavanone intake. Intakes of other major sources of flavanones, namely oranges, orange juice, and grapefruit, were not significantly associated with incident diabetes. Important to note is that a major source of anthocyanins in this cohort was fruit squash/cordial, which has a high sugar content. The co-occurrence of dietary components that may increase type 2 diabetes risk within flavonoid-rich foods and beverages, such as sugar in the previous instances, may explain the U-shaped associations observed for the anthocyanin and flavone subclasses and compounds within those subclasses. Interestingly, although malvidin belongs to the anthocyanin subclass, we observed an inverse association between malvidin intake and diabetes. In this cohort, it is likely that the primary dietary source of malvidin was red wine. A moderate intake of red wine is associated with a lower risk of type 2 diabetes in numerous cohort studies, and red wine has been shown to improve insulin sensitivity, an effect not attributable to its alcohol content, in randomized controlled trials (35). In the current study, apples and tea were likely major contributors to total flavonoid intake (31). In a meta-analysis of 16 cohort studies, higher tea intakes were associated with a lower risk of type 2 diabetes (36). The current study exemplifies the need to interpret associations between dietary sources of flavonoids and health outcomes, in observational studies, in the context of the whole food matrix. It may be that the differing associations for flavonoid subclass intakes and diabetes between studies are due to differing dietary patterns and key sources of flavonoids in the different populations.

Risk factors for type 2 diabetes include smoking (37) and obesity (38). Although associations between total flavonoid intakes and diabetes were present in all subpopulations investigated, the difference (flavonoid intake quintile 5 – quintile 1) in the 20-y estimated absolute risk of diabetes was greatest for those with a BMI ≥30 (men: 4.30%; women: 3.57%), likely due to the higher prevalence of diabetes in this "at-risk" subgroup. Thus, if the association between higher flavonoid intakes and lower diabetes risk is truly causal, ensuring adequate intakes of flavonoid-rich foods in obese individuals will translate to a greater reduction in cases of type 2 diabetes at a population level.

Unlike smoking, obesity may be influenced by flavonoid intake (9) and, therefore, may be an intermediate on the causal pathway between flavonoid intake and diabetes. In randomized controlled trials, short-term ingestion of green tea extract (39), powdered black tea (40), and cocoa or dark chocolate (≥30 g/d) (41), all rich in flavonoids, has been shown

to reduce body weight and improve body fat distribution. In a study of 2734 healthy female twins from the TwinsUK registry, higher habitual intakes of anthocyanins, flavanols, flavonols, and proanthocyanidins were associated with lower fat mass, independent of genetic and environmental factors (13). Furthermore, in a pooled analysis of 3 large prospective cohorts, a higher habitual intake of flavonoids (with the exception of flavones and flavanones) was associated with less weight gain during follow-up, with the strongest inverse associations observed for intakes of anthocyanins and flavanol polymers (14). In the current study, persons with the highest total flavonoid intakes had a 1.52-kg lower body fat at baseline; body fat explained 57% of the total association between total flavonoid intake and incident diabetes. In this cohort, a 1.52-kg lower body fat translated to a 12% lower risk of diabetes. These findings suggest that a protective effect of flavonoids against diabetes may be mediated, in part, by the effect of flavonoids on the metabolism of fat in the body.

There are numerous purported mechanisms by which flavonoids and their metabolites reduce obesity and type 2 diabetes incidence, including enhancing insulin secretion and reducing insulin resistance, potentially by decreasing apoptosis and promoting proliferation of pancreatic β-cells, and reducing inflammation and oxidative stress in muscle (8, 9). A meta-analysis of randomized controlled trials concluded that green tea consumption significantly reduces fasting glucose and hemoglobin A1c concentrations (42), whereas flavonoid-rich chocolate or cocoa consumption significantly reduces insulin resistance and fasting insulin (43). However, the exact mechanisms remain to be elucidated (44); findings from preclinical studies are often not replicated in human studies (45) likely due to limited studies on flavonoid metabolites to date. Evidence for the antidiabetic effects of a diet rich in flavonoids is growing. Despite this, there has been minimal translation of this research into either policy or practice. This is likely due, at least in part, to an incomplete understanding of the mechanisms and pathways linking flavonoid intake to health benefits (46). Complete characterization is challenging because bioavailability and bioactivity differ between flavonoid compounds, and both phase 2 and gut-derived flavonoid metabolites likely also exert biologically relevant effects (47).

Conclusions

In this Danish prospective cohort study, we observed that higher flavonoid intakes were cross-sectionally associated with lower estimates of body fat and longitudinally associated with a lower risk of diabetes, particularly for the flavonol, flavanol monomer, and flavanol oligo + polymer subclasses. Our results suggest that promoting a diet abundant in healthy flavonoid-rich foods may ameliorate diabetes risk, in part through a reduction in body fat.

Data Availability

The data, codebook, and analytic code will be made available upon request pending application and approval by the Diet, Cancer, and Health Steering Committee at the Danish Cancer Society.

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