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Association between non-tea flavonoid intake and risk of type 2 diabetes: the Australian diabetes, obesity and lifestyle study†

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Studies examining the association between flavonoid intake and measures of insulin resistance and β-cell dysfunction, as markers of type 2 diabetes (T2DM) across the adult lifespan, may provide insights into how flavonoids influence T2DM risk. This study examined the cross-sectional associations between flavonoid intakes, from dietary sources other than tea, and biomarkers of glucose tolerance and insulin sensitivity in adults aged 25 years and older participating in the Australian diabetes, obesity and lifestyle (AusDiab) study. Additionally, longitudinal associations between non-tea flavonoid intakes and incident T2DM over 12 years were explored. Eligible participants (n = 7675) had no previous history of T2DM and had completed a food-frequency questionnaire at baseline (1999–2000) from which flavonoid intakes were calculated using United States Department of Agriculture Databases. Restricted cubic splines in regression models were used to examine cross-sectional associations between intakes of total non-tea flavonoids and selected flavonoid subclasses and measures of glucose tolerance and insulin sensitivity including glycated haemoglobin (HbA1c), homeostasis model assessment of β-cell function (HOMA2-% β) and insulin sensitivity (HOMA2-% S), 2-hour post load plasma glucose (PLG), fasting plasma glucose (FPG) and fasting insulin levels. Associations between flavonoid intakes and T2DM risk were estimated using Cox proportional hazards models. Cross-sectionally, significant beneficial associations were observed for intakes of total flavonoids and the flavan-3-ol-monomer, proanthocyanidin, flavonol and anthocyanidin subclasses with measures of glucose tolerance and insulin sensitivity ($P < 0.05$ for all), except fasting plasma glucose. During follow-up, 344 incident T2DM cases were recorded. Participants with the highest total flavonoid intake had a 21% lower risk of T2DM over 12 years, although this was not statistically significant in multivariable adjusted models [HR (95% CI): 0.79 (0.57, 1.09)]. This study provides some evidence that consuming flavonoid-rich foods may be protective against T2DM through mechanisms related to glucose tolerance and insulin sensitivity.

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Introduction

Impaired insulin secretion (β-cell dysfunction) and increased insulin resistance play an important role in the development of type 2 diabetes mellitus (T2DM).[1] Due to the associated micro and macro vascular complications, T2DM is recognised to have a considerable impact on morbidity, mortality and health expenditure.[2] The role of diet in the etiology of T2DM is well established,[3] and evidence shows that a diet high in flavonoids[4,5] and low in refined grains, alcohol and processed meat[4,6] is associated with a 0.53–0.89 fold lower risk of developing diabetes.

Flavonoids are bioactive plant metabolites found in foods and beverages including tea, fruits, red wine, chocolate and vegetables.[7] Based on their chemical structure, flavonoids are generally categorised into six major subclasses (flavonols, flavan-3-ols, flavones, flavanones, anthocyanins and isoflavones) with certain subclasses being abundant in specific foods.[8,9] We have previously reported a non-linear inverse association between flavonoid intake and T2DM in the Danish Diet Cancer and Health cohort of men and women aged 50 to 64 years.[10] A significant inverse association has also been observed between intakes of flavones, flavanols, flavanones and anthocyanins and T2DM risk in the health, alcohol and psychosocial factors in Eastern Europe cohort of adults aged 45 to 69 years.[11]

Studies examining the association between flavonoid intake and measures of insulin resistance and β-cell dysfunction as markers of T2DM, may further provide insights into how flavonoids influence T2DM risk, but these are scarce. In a cross-sectional study in male Koreans aged 40 to 59 years, intakes of flavonols and flavones from tea and other flavonoid rich foods were inversely correlated with insulin resistance.[12] Data from the United Kingdom (UK) Twins registry revealed that higher intakes of anthocyanins and flavones in female twins aged 18 to 76 years were significantly associated with lower insulin resistance and fasting insulin levels.[13] Furthermore, a recent review of clinical trials and epidemiological studies indicated that anthocyanins and flavan-3-ols rich foods may play an important role in glucose absorption and insulin sensitivity and/or secretion.[14]

Tea is a major contributor to flavonoid intake in many populations and has been reported to contribute between 58–80% of total flavonoid intake in Australian cohorts.[15–17] As such, in observational studies, total flavonoid intake estimates may simply be a reflection of tea intake, where its inverse association with T2DM is well-established.[18] Tea intake was not captured in the Australian diabetes, obesity and lifestyle cohort but of interest is the association of non-tea flavonoid-rich foods and beverages with T2DM risk which to our knowledge has yet to be explored. Furthermore, most previous studies have often focus on older adults[10–12] and have only considered a few markers of T2DM.[12,13] Thus, there is a need for a more detailed exploration of the relationship between flavonoid intake, particularly from non-tea sources, and diverse and clinically relevant measures of insulin resistance and β-cell dysfunction as well as incidence of T2DM. Therefore, the aims of this study were to examine the association between total flavonoid intake and individual flavonoid subclasses from non-tea dietary sources in a cohort of Australian men and women aged 25 years and over with (i) cross-sectional measures of insulin resistance and β-cell dysfunction and (ii) the risk of developing T2DM over 12 years.

Methods

Study population

Participants in this study were from the Australian diabetes, obesity and lifestyle (AusDiab) study for which full methodology and response rates are described elsewhere.[19,20] Briefly, this national study examined the prevalence of diabetes mellitus and its associated risk factors in men and women aged ≥25 years, recruited from the six states and the Northern Territory of Australia. Baseline measurements were collected in 1999–2000 (n = 11 247) and participants were followed-up at 5 years (2004–2005; n = 6400) and 12 years (2011–2012; n = 4614). In the current study, a total of 3572 participants were excluded at baseline for the following reasons: implausible energy intakes (<3300 kJ day^{-1} or >17 500 kJ day^{-1} for males and <2500 kJ day^{-1} or >14 500 kJ day^{-1} for females)[21] (n = 342), pregnancy (n = 45), incomplete food frequency questionnaire (FFQ) (n = 204), missing outcome (n = 1371) and baseline covariate data (n = 642). We further excluded 986 individuals with prevalent diabetes at baseline (ESI Fig. 1†). The study protocol was approved by the Human Research Ethics Committees of the International Diabetes Institute, Alfred Hospital, and by the International Diabetes Institute Ethics Committee (Melbourne, Australia).

Dietary assessment

Dietary intake of participants was assessed using a validated semi-quantitative 74-item FFQ developed by the Victorian Anti-Cancer Council of Australia.[22,23] The FFQ measures participants' frequency of food items consumption over the past 12 months with 10 possible response options ranging from "never" to "three or more times per day". These results were adjusted according to global questions regarding frequency of intake to reduce intake over-estimation. Frequency data was multiplied by portion sizes, which were calculated using photographs of scaled portions of different food types, to estimate intakes in g day^{-1}. Tea intake was not captured in the version of the FFQ used in this study. Energy and nutrient intake calculations were analysed by the Victorian Anti-Cancer Council using the Australian Food Composition Tables (NUTTAB95), based on frequency of consumption and an overall estimate of usual portion sizes. These estimates were supplemented by other databases where necessary.

Exposures

The exposures of interest for this study were baseline intakes of total flavonoids and the flavonoid subclasses with mean

intakes of ≥5 mg day^{-1}, calculated by multiplying the estimated daily consumption (g edible portion per day) by the flavonoid subclass content of each food item as done previously.[24] The flavonoid content for each food item was obtained from the United State Department of Agriculture Comprehensive Flavonoid 3.3 (2018), Isoflavone (2015) and Proanthocyanidin (2018) contents of foods databases.[7,25,26] For each food item, the intake of each flavonoid compound was estimated. Total intake of each subclass (flavonols, flavan-3-ols monomers, proanthocyanidins, anthocyanins, flavones, flavones and isoflavones) were determined by summing the individual flavonoid compounds within each subclass. Total flavonoid intake was then calculated by summing each of the flavonoid subclasses. Items in the FFQ that were not found in the USDA flavonoid databases were assumed to have a flavonoid content of zero.

Study outcomes

Cross-sectional outcomes of interest were measures of glucose tolerance, insulin sensitivity and β-cell activity including 2-hour post load plasma glucose (PLG), homeostasis model assessment (HOMA2) of β-cell function (HOMA2-% β), HOMA2 of insulin sensitivity (HOMA2-% S), fasting plasma glucose (FPG), glycated haemoglobin (HbA1c) and fasting insulin levels at baseline. Details of measurement techniques are previously described.[19] Briefly, PLG and FPG were determined by a spectrophotometric-hexokinase method while HbA1c was analysed using high-performance liquid chromatography method[27] and serum insulin was measured with an automated chemiluminescence immunoassay.[28] The HOMA2 computer model from physiological dose responses of glucose uptake and insulin production was used to estimate β-cell function (HOMA2-% β) and insulin sensitivity (HOMA2-% S); this approach has been used widely in previous epidemiological studies.[29,30] The longitudinal outcome was incidence of T2DM over 12 years of follow-up. T2DM was classified according to the World Health Organisation Criteria of FPG ≥7.0 mmol L^{-1}, 2-hour PLG ≥11.1 mmol L^{-1}, HbA1c ≥6.5% or current treatment with oral hypoglycaemic medications or insulin.[31]

Covariates

At baseline, socio-demographic data were collected using an interviewer administered questionnaire. These include age, sex (male/female), smoking status (current/former/never), income and education level (never/some high level; completed university or equivalent). Physical activity level was categorised as no activity: 0 min per week; insufficient: 1 to 150 min per week; and sufficient: ≥150 min per week. The socio-economic indexes for areas (SEIFA) as reported by the Australian Bureau of Statistics[32] was obtained as well as parental history of diabetes (yes/no) and self-reported history of cardiovascular disease [angina, heart attack and stroke] (yes/no). All dietary confounders were obtained from the FFQ data and other details of questionnaire have been discussed elsewhere.[19] In addition, physical examination includes anthropometric measurement of body weight and height which were used to derive BMI.

Statistical analysis

A prespecified analytic protocol was developed before commencing statistical analysis. All cross-sectional outcomes were positively skewed, therefore generalised linear models with a gamma distribution and log-link were used to examine the associations between exposure variables and these outcomes. Non-linear associations were modelled with restricted cubic splines (internal knots at the 0.275, 0.5 and 0.725 percentiles; external knots at the 0.05 and 0.95 percentiles allowing flexibility to model non-linear relationships between continuous variables and outcomes in regression models[33] which were hypothesised based on previous studies.[4,10] Cross-sectional associations are presented graphically using the 'effects' R package.[33] Likelihood ratio tests comparing appropriate nested models were used to examine the overall effect of the exposure on the response (false discovery rate corrected) and to test-for non-linearity. Ratio of means and 95% confidence intervals (CI) were obtained from each model with exposure fitted as a continuous variable through the restricted cubic spline using the 'rms' R package.[34] Ratio of means and 95% CI for the median in each quartile (Q2–Q4) relative to the median intake in the lowest quartile (Q1) are presented to demonstrate where significant differences between quartiles of intake exist.

Multivariable Cox proportional hazard models were used to investigate relationships between flavonoid intake and incident T2DM with age as the underlying time scale.[35] Age at study exit was calculated from each participants' date of birth up until the date of a first-time diagnosis of T2DM, loss to follow-up, death, or the end of the study, whichever came first. The date of T2DM diagnosis or loss to follow-up was taken as the mid-point between follow-ups. Using the 'rms' R package, hazard ratio (HRs) and 95% CIs were calculated from the model by fitting the exposures as continuous variables with restricted cubic splines. HR estimates are graphed over a fine grid of x values with the axis truncated at 3 SDs above the mean for visual simplicity.

In all analyses, the median intake of Q1 was chosen as the reference value and estimates are reported for the median of each subsequent quartile. Three models of adjustment were adopted in all regression analyses; model 1 adjusted for age (years) and sex (male/female); model 2 adjusted for age, sex, level of education (never to some high schools, completed university or equivalent); physical activity levels (sedentary, insufficient, sufficient), income, smoking status (current smoker, ex-smoker, non-smoker), SEIFA, BMI (kg m^{-2}), parental history of diabetes (yes/no) and self-reported prevalence of cardiovascular disease (yes/no); model 3 adjusted for all covariates in model 2 in addition to energy intake and intakes (g day^{-1}) of beer, spirits, red meat and processed meat. Covariates were chosen *a priori* to the best of our knowledge of potential confounders of flavonoid intake and incidence of T2DM. Statistical analyses were performed using STATA/IC 16.1 (StataCorp LLC) and R statistics (R Core Team, 2020 [36]).

Results

At baseline, there were 7675 participants (45% male) with a median age of 52 (IQR: 44–62, range: 36–91) years. There was ~4-fold difference in total flavonoid intake between participants in the highest (Q4) and lowest (Q1) quartiles (median: 325 mg d^{-1} *vs.* 72 mg d^{-1}). Compared to participants with the lowest flavonoid intakes, those with the highest intakes tended to have a higher socio-economic and educational status as well as healthier lifestyle habits as they were less likely to be current smokers, more physically active and had higher intake of vegetables. Participants across all quartiles had similar frequencies of prevalent CVD and family history of T2DM as well as related patterns of processed and red meat consumption (Table 1).

The median daily intakes (mg d^{-1}) of total flavonoids and each flavonoid subclass are presented in ESI Table 1.† Proanthocyanidins (median; IQR: 101.8; 60.1–157.8), flavan-3-ols monomer (median; IQR: 17.5; 10.7–26.4) and anthocyanins (median; IQR: 12.6; 6.3–24.7) were the three highest contributors to total flavonoid intake. The main dietary sources of total flavonoid were fruits, vegetables, chocolate products and red wine.

Cross-sectional associations between total flavonoid intake and measures of β-cell dysfunction and insulin resistance

Significant inverse associations were observed between total flavonoid intakes and HbA1c, serum insulin, HOMA2-% β and PLG levels while a positive association was observed for HOMA2-% S (Fig. 1; false discovery rate corrected $P \leq 0.05$ for all). Compared to participants with the lowest total flavonoid intakes (Q1), participants with the highest intakes (Q4) had a 2% lower HbA1c (ratio of means [95% CI]: 0.98 [0.98, 0.99]), 6% lower serum insulin (0.94 [0.92, 0.97]), 4% lower HOMA2-% β (0.96 [0.94, 0.97]), 4% lower PLG (0.96 [0.95, 0.98]), and 7% higher HOMA2-% S (1.07 [1.04, 1.10]) (model 2; ESI Table 2†). Adjusting for potential dietary confounders did not substantially alter these associations (model 3; ESI Table 2†). Similar associations were observed for intakes of flavan-3-ol monomer, proanthocyanidin, flavonol and anthocyanin subclasses and HbA1c, serum insulin, HOMA2-% β and PLG outcomes (model 2 and model 3; ESI Table 2 and Fig. 2–5;† false discovery rate corrected $P \leq 0.05$ for all). There was some evidence that flavanone intakes were inversely associated with HOMA2-% β and positively associated with HOMA2-% S (ESI Fig. 6†).

Prospective associations between total flavonoid and flavonoid subclasses intake and incidence of type 2 diabetes up to 12 – years follow-up

During 12 years of follow-up, 344 cases of T2DM were recorded. HRs (95% CIs) for incident T2DM by quartiles of total flavonoid intake and intake of flavonoid subclasses are shown in Table 2. Baseline total flavonoid, flavan-3-ol and

Table 1 Baseline characteristics of the study population

	All participants (n = 7675)	Total non-tea flavonoid intake quartiles			
		Q1 (n = 1919)	Q2 (n = 1919)	Q3 (n = 1918)	Q4 (n = 1919)
Total non-tea flavonoid intake (mg d^{-1}), median (IQR)	165 (103–248)	72 (52–89)	134 (118–149)	202 (184–222)	325 (248–406)
Socio-demographics					
Sex, male, n (%)	3439 (44.8)	681 (35.5)	736 (38.4)	865 (45.1)	1157 (60.3)
Age, years, median (IQR)	52 (44–62)	52 (44–63)	51 (44–62)	52 (44–63)	51 (44–61)
BMI	26.8 ± 4.7	26.9 ± 4.9	26.9 ± 4.8	26.7 ± 4.6	26.7 ± 4.3
SEIFA score, median (IQR)	1033 (972–1079)	1004 (960–1065)	1033 (972–1075)	1045 (974–1081)	1050 (996–1083)
Smoking status, *n* (%)					
Current	1097 (14.3)	392 (20.4)	284 (14.8)	215 (11.2)	206 (10.7)
Former	2319 (30.2)	537 (27.9)	531 (27.7)	574 (29.9)	677 (33.3)
Never	4259 (55.5)	990 (51.6)	1104 (57.5)	1129 (58.9)	1036 (54.0)
Education *n* (%)					
Never, primary or high school	3114 (40.6)	940 (48.9)	844 (43.9)	715 (37.3)	615 (32.1)
Secondary education or higher	4561 (59.4)	979 (51.0)	1075 (56.0)	1203 (67.7)	1304 (67.9)
Physical activity, *n* (%)					
Sedentary	1308 (17.0)	466 (24.3)	343 (17.9)	271 (14.1)	228 (11.9)
Insufficient	2377 (30.9)	629 (32.8)	618 (32.2)	628 (32.7)	502 (26.2)
Sufficient	3990 (51.9)	824 (42.9)	958 (49.9)	1019 (53.1)	1189 (61.9)
Family history of diabetes, n (%)	1366 (17.8)	350 (18.2)	362 (18.9)	341 (17.8)	313 (16.3)
Prevalent CVD, n (%)	609 (7.9)	165 (8.6)	165 (8.6)	137 (7.1)	142 (7.4)
Dietary characteristics, median (IQR)					
Total energy intake, kJ	7846 (6164–9858)	6467 (5163–8095)	7272 (5855–9815)	8169 (6654–10 057)	9643 (7949–11 704)
Sugar intake, g d^{-1}	87 (67–112)	68 (51–85)	81 (64–99)	94 (75–114)	114 (90–140)
Vegetable intake, g d^{-1}	162.3 (117.9–217.6)	138.9 (99.4–189.7)	156.8 (112.9–205.7)	164.6 (123.4–218.7)	192.1 (143.2–251.4)
Alcohol intake, g d^{-1}	5.7 (0.6–18.5)	1.7 (0.3–8.7)	4.1 (0.5–13.9)	7.4 (1.0–19.2)	14.2 (2.3–30.5)
Red meat, g d^{-1}	59 (34–96)	52 (28–85)	55(32–88)	59(61–98)	74(45–1122)
Processed meat, g d^{-1}	17 (8–31)	15 (7–28)	16 (8–31)	17 (7–32)	20 (8–36)

Abbreviations: CVD, cardiovascular disease; IQR, interquartile range; Q, quartile; SEIFA, socio-economic index for areas.

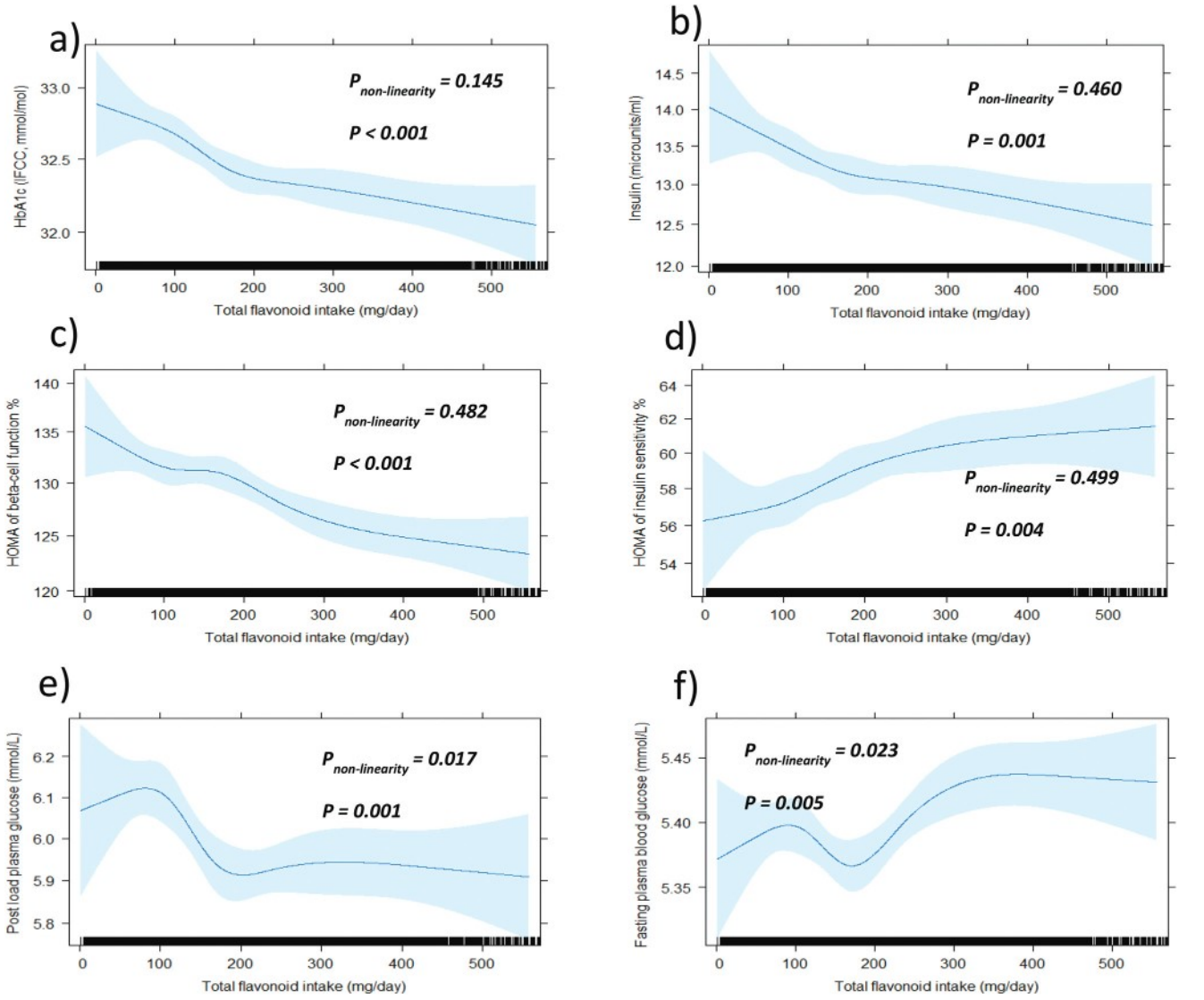

Fig. 1 Graphical representation of the multivariable-adjusted dose–response relationship between total flavonoid intake and baseline (a) glycated haemoglobin, (b) fasting serum insulin, (c) HOMA2 of β-cell function, (d) HOMA2 of insulin sensitivity, (e) 2-hour post-load plasma glucose and (f) fasting plasma glucose, obtained by generalised regression models with exposure included as a restricted cubic spline (n = 7675). HOMA of β-cell function and HOMA of insulin sensitivity were estimated using HOMA2 computer model. The blue shaded areas represent 95% confidence intervals. The distribution of the exposure variable is provided in the rug plot on the x-axis of each graph. All analyses were adjusted for age, sex, level of education, physical activity levels, income, smoking status, socio-economic index for areas (SEIFA), BMI, parental history of diabetes, prevalence of cardiovascular disease and intakes of beer, spirits, red meat, processed meat, and energy. P-Values for the association between exposure and outcomes (false discovery rate corrected) and for tests of non-linearity were obtained using likelihood ratio tests.

proanthocyanidin intakes were non-linearly inversely associated with incident diabetes (Fig. 2). However, estimates were not statistically significant in multivariable-adjusted models and hazard ratios were higher when dietary confounders were included in the models (Table 2). Compared to those with the lowest intakes (Q1), participants with the highest intakes (Q4) of total flavonoids, flavan-3-ols and proanthocyanidins had a non-significant 21% [HR (95% CI): 0.79 (0.57, 1.09)], 14% [HR (95% CI): 0.86 (0.62, 1.12)], and 20% [HR (95% CI): 0.80 (0.58, 1.10)] lower risk of T2DM over 12 years, respectively, after multivariable adjustments (model 3; Table 2). No clear associations were observed for intakes of flavonols, flavanones and anthocyanins (Fig. 2 and Table 2).

Discussion

The main findings from this study were that higher intakes of total flavonoids and the flavan-3-ol-monomer, proanthocyanidin, flavonol and anthocyanin subclasses, from non-tea dietary sources, were associated with better measures of β-cell function and insulin sensitivity except fasting plasma glucose, in

Table 2 Cox proportional hazard ratios (95% CIs) for risk of incident type 2 diabetes p to 12 years by intakes of total flavonoid and flavonoid subclasses (n = 7675)

	Flavonoid intake quartiles			
	Q1	Q2	Q3	Q4
Total flavonoids				
Model 1	Ref.	0.82 (0.67, 1.01)	0.69 (0.55, 0.87)	0.63 (0.47, 0.83)
Model 2	Ref.	0.88 (0.71, 1.08)	0.80 (0.63, 1.01)	0.76 (0.57, 1.02)
Model 3	Ref.	0.88 (0.71, 1.09)	0.81 (0.63, 1.04)	0.79 (0.57, 1.09)
Flavan-3-ols				
Model 1	Ref.	0.78 (0.63, 0.95)	0.71 (0.57, 0.89)	0.67 (0.50, 0.90)
Model 2	Ref.	0.82 (0.67, 1.01)	0.82 (0.65, 1.03)	0.83 (0.62, 1.12)
Model 3	Ref.	0.82 (0.66, 1.02)	0.83 (0.65, 1.06)	0.86 (0.62, 1.12)
Proanthocyanidins				
Model 1	Ref.	0.78 (0.64, 0.96)	0.69 (0.55, 0.86)	0.65 (0.49, 0.87)
Model 2	Ref.	0.83 (0.67, 1.02)	0.78 (0.62, 0.98)	0.77 (0.58, 1.04)
Model 3	Ref.	0.83 (0.67, 1.02)	0.79 (0.62, 1.01)	0.80 (0.58, 1.10)
Flavonols				
Model 1	Ref.	0.84 (0.70, 1.02)	0.77 (0.62, 0.97)	0.77 (0.58, 1.04)
Model 2	Ref.	0.88 (0.72, 1.07)	0.86 (0.68, 1.09)	0.90 (0.66, 1.21)
Model 3	Ref.	0.89 (0.73, 1.09)	0.89 (0.70, 1.14)	0.95 (0.67, 1.35)
Anthocyanins				
Model 1	Ref.	0.95 (0.76, 1.19)	0.94 (0.72,1.21)	0.91 (0.67, 1.22)
Model 2	Ref.	0.99 (0.79, 1.25)	1.06 (0.82, 1.38)	1.15 (0.84, 1.56)
Model 3	Ref.	1.00 (0.80, 1.26)	1.09 (0.83, 1.43)	1.21 (0.87, 1.67)
Flavanones				
Model 1	Ref.	1.19 (0.94, 1.51)	0.92 (0.71, 1.20)	0.64 (0.47, 0.87)
Model 2	Ref.	1.28 (1.01, 1.64)	1.07 (0.82, 1.40)	0.77 (0.57, 1.05)
Model 3	Ref.	1.29 (1.01, 1.65)	1.09 (0.83, 1.43)	0.80 (0.58, 1.10)

Hazard ratios are reported for the median intake in each quartile relative to the median intake in quartile 1. Model 1 adjusted for age and sex; model 2 adjusted for age, sex, smoking status, SEIFA (socio-economical index for areas), physical activity levels, education, income, BMI, parental history of diabetes and self-reported prevalence of cardiovascular disease; model 3 adjusted for all covariates in model 2 in addition to energy intake, and intakes (g day^{-1}) of beer, spirits, red meat, and processed meat.

Australian adults aged 25 years and over. Over 12 years of follow-up, higher baseline intakes of flavonoids, flavan-3-ols and proanthocyanidins were non-significantly associated with a 14 to 21% lower risk of developing T2DM in the fully adjusted model.

Although associations between total flavonoid, flavan-3-ol and proanthocyanidin intakes and incident T2DM were not statistically significant, the hazard ratios observed (0.79, 0.86 and 0.80, respectively) were the same or even lower than the relative risks (RR) reported previously in meta-analysis from 2018 of 10 prospective cohorts (312 015 participants and 19 953 incident diabetes cases identified between 4 and 28 years of follow-up).[5] Here, the highest intakes of total flavonoids were associated with an 11% lower risk of T2DM (RR: 0.89, 95% CI: 0.82, 0.96), when compared to the lowest intakes.[5] For flavonoid subclasses, the meta-analysis reported a 14% lower risk of T2DM for the highest *versus* lowest intakes of flavan-3-ols (RR: 0.86, 95% CI: 0.78, 0.95) and flavonols (RR: 0.86, 95% CI: 0.80, 0.94) and a non-significant 12% lower risk for proanthocyanidins (RR: 0.88, 95% CI: 0.75, 1.02).[5] The findings that the associations were not statistically significant in the present study may be due to low statistical power, owing to our low number of events, or due to our lack of data on date of diabetes diagnosis. However, important to note is that the food sources of flavonoids in all the aforementioned studies included tea, limiting comparison and generalisation.

To our knowledge, observational studies exploring the associations between non-tea flavonoid intake and biomarkers of T2DM as assessed in the current study are lacking[12,13] and none have examined associations between flavonoid intake and a comprehensive panel of T2DM biomarkers or association with incident diabetes across the adult lifespan. Our findings corroborate previous findings of an inverse association between intakes of flavonols[12] and anthocyanins[13] with insulin resistance, and further extend the evidence base by demonstrating clear inverse associations between flavonoid intake and HbA1c, HOMA2 of β-cell function and post-load plasma glucose. These associations are supported by findings from randomised controlled trials which uses flavonoid food sources of cocoa, dark chocolate, and vegetables in short term interventions and reported better levels of HbA1c, insulin resistance and beta-cell function.[37–39] Among flavonoid subclasses, we observed no significant cross-sectional associations for higher intakes of flavanones and the outcomes of HbA1c, serum insulin and post load glucose. Furthermore, the longitudinal association was unclear as the risk was higher at lower intakes. This finding is consistent with other observational studies,[5,10,11] but in contrast with many *in vivo*, animal studies and human clinical trials which have reported protective effect of flavanones on the risk of T2DM.[40–42] However, it is important to note that in the trials, the amount of flavonoids provided was much higher than is naturally achievable *via* diet sources.[42]

In the present study, higher flavonoid intakes were inversely associated with β-cell function and positively associated with insulin sensitivity in a dose–response manner. Regarding the first association, important to note is that the HOMA2 of β-cell function measurement estimates β-cell activity and a decline in activity reflects better sensitivity, where β-cells do not need to secrete as much insulin because the cells are more sensitive to its effect.[29] In the current study, the higher insulin sensitivity seen with a higher intake of flavonoids appeared to translate to lower post-load glucose and HbA1c levels.

There are several mechanisms hypothesised to explain the observed cross-sectional associations in this study. These include the ability of dietary flavonoids to reduce insulin resistance in insulin-sensitive tissues through the activation of glucose uptake and the regulation of insulin secretion from pancreatic β-cells to maintain glucose homeostasis.[43] Studies have highlighted the role of anthocyanins, flavan-3-ols and flavonols in improving glycemia metabolism, insulin resistance and β-cell dysfunction.[44] Furthermore, flavonoids are regulators of lipid profiles and oxidative stress, which are linked to the prevention of T2DM.[45] Additionally, it has been reported that flavonoids influence the composition of the gut microbiome which plays a major role in the pathophysiology of T2DM and its biomarkers.[46]

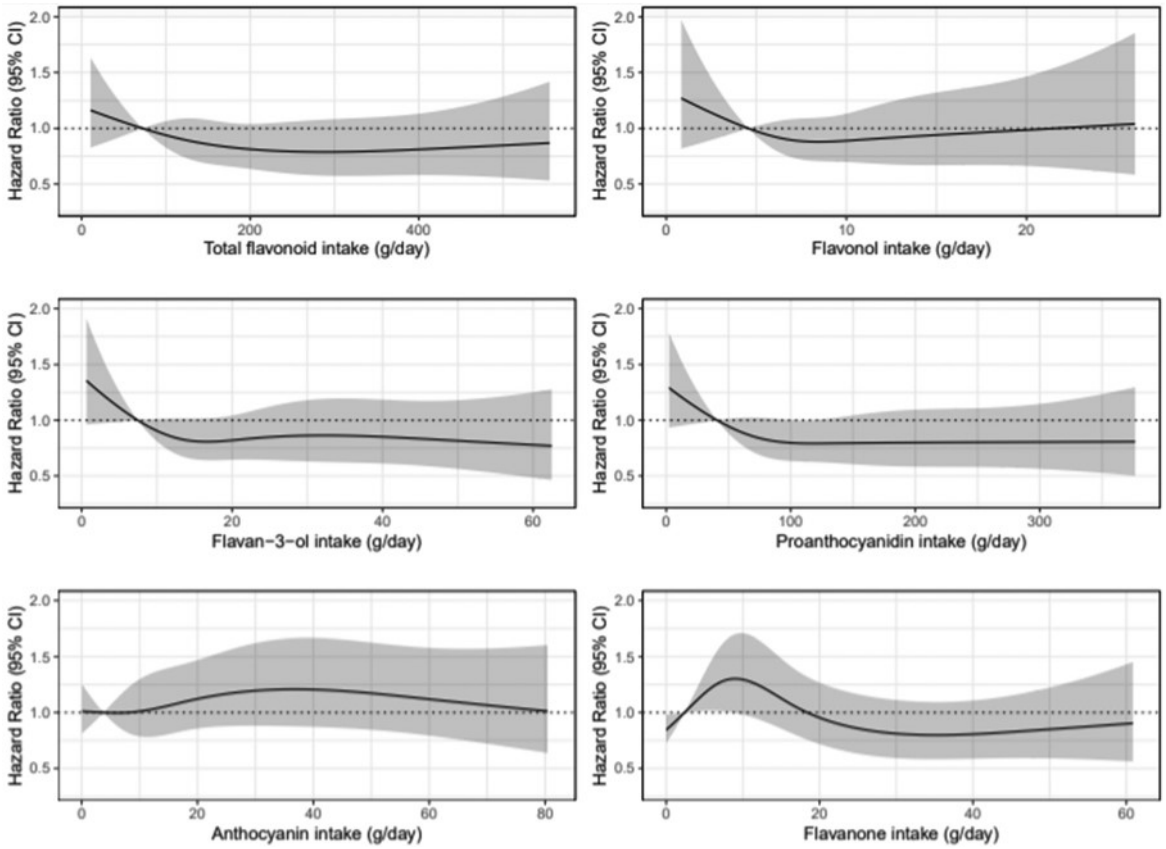

Fig. 2 Cubic spline curves describing the association between total and individual flavonoid subclass intakes and incident type 2 diabetes mellitus over 12 years (n = 7675). Hazard ratios and 95% confidence intervals are based on Cox proportional hazards models comparing the specific level of flavonoid intake (x-axis) to the median intake for participants in the lowest intake quartile. All analyses are adjusted for age, sex, level of education, physical activity levels, income, smoking status, socio-economic index for areas (SEIFA), BMI, parental history of diabetes, prevalence of cardiovascular disease and intakes of beer, spirits, red meat, processed meat, and energy (model 3).

The strengths of this study relate to the investigation of the association between a range of flavonoid subclasses and an extensive panel of biomarkers of insulin sensitivity and glucose metabolism. Only a few epidemiological studies have explored these relationships and typically these studies have investigated only one or two biomarkers.[12,47] The assessment of several biomarkers that partly reflect different mechanistic pathways, facilitates a better understanding of the potential effects of flavonoids in relation to T2DM. Other strengths include the prospective design, large sample size from a national cohort of Australian adults across a broad age range with long follow-up period.

While this study contributes a focussed assessment of the role of non-tea flavonoid intake on T2DM and its biomarkers, it is not without limitations. Tea intake was not captured in the FFQ used and therefore flavonoid intakes could only be estimated from non-tea dietary sources. However, in a large population study in Denmark, participants with a high tea intake also have a higher intake of fruit and vegetables, other key dietary sources of flavonoids.[48] Therefore, it is likely that those with low intakes of non-tea flavonoids in the present study also have a low intake of tea. Furthermore, this study analysed flavonoid containing foods that also contain other possible protective phytochemicals, which could contribute to the observed beneficial effects, possibly in an interdependent or complementary manner. In addition, the observed associations might be influenced by other lifestyle confounders since in most populations and as seen in this study, high flavonoid intake is a strong indicator of a healthier lifestyle. Although, we adjusted for other indicators of healthier lifestyles in the analysis, possible residual confounders cannot be ruled out.

Conclusion

In conclusion, this study provides a comprehensive investigation of associations between intakes of total flavonoids and major flavonoid subclasses and both measures of glucose tolerance and insulin sensitivity and the development of T2DM. Our results provide some evidence that consuming flavonoid-rich foods may be protective against T2DM through mechanisms related to glucose tolerance and insulin sensitivity.

Abbreviations

AusDiab	Australian diabetes obesity and lifestyle study
BMI	Body mass index
CI	Confidence interval
FFQ	Food frequency questionnaire
FPG	Fasting plasma glucose
HbA1c	Glycated haemoglobin
HOMA2-	Updated homeostasis model assessment of insulin resistance
HOMA2-% β	Updated homeostasis model assessment of cell-function
HOMA2-% S	Updated homeostasis model assessment of insulin sensitivity
HR	Hazard ratio
PLG	Post load plasma glucose
Q	Quartile
SEIFA	Socio-economic indexes for areas
T2DM	Type 2 diabetes mellitus

Data availability

Our researchers were granted access to the study data from the AusDiab steering committee under a licensing agreement. Requests for its use and access for research are made possible through contact with the steering committee of AusDiab.

Author contributions

MDA, NB, CB, MS, JH, RD, JRL, DJM and JS designed the research; NB, CB and BP curated the data; MDA and NB analysed the data; DJM, RMD and JES conducted the original cohort study; MDA wrote the manuscript and had primary responsibility for all content; All authors assisted with results interpretation and critically reviewed the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study, data analyses nor interpretation, writing of the manuscript or in the decision to publish the results.

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