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Bamini Gopinath

Gerald Liew

Annette Kifley

Victoria M. Flood

Nichole Joachim

See next page for additional authors

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Dietary flavonoids and the prevalence and 15-year incidence of age-related macular degeneration

Running title: Flavonoids and age-related macular degeneration

Bamini Gopinath[1]

Gerald Liew[1]

Annette Kifley[1]

Victoria M Flood[2,3]

Nichole Joachim[1]

Joshua R Lewis[4,5,6]

Jonathan M Hodgson[5,6]

Paul Mitchell[1]

[1]Centre for Vision Research, Department of Ophthalmology and Westmead Institute for Medical Research, University of Sydney, NSW, Australia.

[2] Faculty of Health Sciences, University of Sydney, Sydney, NSW, Australia.

[3] Westmead Hospital ,Western Sydney Local Health District, Westmead, NSW, Australia

[4] Centre for Kidney Research, Westmead, School of Public Health, University of Sydney, NSW, Australia.

[5] School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia.

[6] School of Medicine, University of Western Australia, 35 Stirling Highway, Perth, WA, Australia.

Names for PubMed indexing: Gopinath; Liew; Kifley; Flood; Joachim; Lewis; Hodgson; Mitchell.

Correspondence to:

Associate Professor Bamini Gopinath
Centre for Vision Research
Westmead Hospital, Westmead, NSW 2145, Australia.
Telephone: 61-2-8627 3368 Fax: 61-2-8627 3099
Email: bamini.gopinath@sydney.edu.au

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Abbreviations

AMD **–** Age-related macular degeneration

AREDS - Age-Related Eye Disease Study

BMES – Blue Mountains Eye Study

FFQ – Food Frequency Questionnaire

GWAS - Genome-Wide Association Study

SNP – Single Nucleotide Polymorphism

USDA - US Department of Agriculture

Abstract

Background: The majority of research performed to date has examined the effects of commonly known antioxidants such as vitamins C, E and A, and carotenoids on age-related macular degeneration (AMD) risk and progression. To date, there is limited research on promising phytochemicals with antioxidant and anti-inflammatory properties, including flavonoids.

Objectives: In this exploratory study, we aimed to assess the independent associations between dietary intake of total flavonoids and common flavonoid classes with the prevalence and 15-year incidence of AMD.

Design: In this population-based cohort study, 2856 adults aged 49+ years at baseline and 2037 followed up 15-years later were included in prevalence and incidence analysis, respectively. Dietary intake was assessed using a semi-quantitative food-frequency questionnaire (FFQ). Estimates of the flavonoid content of foods in the FFQ were assessed using the US Department of Agriculture Flavonoid, Isoflavone and Proanthocyanidin databases. AMD was assessed from retinal photographs.

Results: In cross-sectional analysis, each 1-SD increase in total overall flavonoid intake was associated with reduced likelihood of any AMD, multivariable-adjusted OR 0.76 (95% CI 0.58, 0.99). Each 1-SD increase in dietary intake of total flavonol and total flavanone was associated with reduced odds of prevalent any AMD: multivariable-adjusted OR 0.75 (95% CI 0.58, 0.97); and OR 0.77 (95% CI 0.60, 0.99), respectively. A marginally significant trend ($p=0.05$) was observed between increasing intake of total flavanone and hesperidin (from first to fourth quartile) and reduced likelihood of incident late AMD, after multivariable adjustment. Participants who reported one or more serves of oranges per day versus those who never ate oranges at baseline had reduced risk of late AMD 15 years later: multivariable-adjusted OR 0.39 (95% CI 0.18, 0.85).

Conclusions: Our novel findings suggest an independent and protective association between dietary intake of flavonoids and the likelihood of having AMD. Additional prospective cohort studies are needed to validate these findings.

Keywords: age-related macular degeneration; flavonoids; Blue Mountains Eye Study; Prevalence; Incidence.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness and severe visual impairment in older adults (1). Current evidence suggests that AMD patients should be given dietary advice to increase consumption of dark green leafy vegetables, to consume low-glycemic index diets, and to consume fish at least twice a week (2-6). The Age-Related Eye Disease Study (AREDS) demonstrated that taking a supplement containing high doses of vitamin C, vitamin E, beta-carotene, zinc, and copper could reduce AMD progression by 25% (7-12). A follow-up study (AREDS 2), found adding lutein and zeaxanthin (naturally occurring carotenoids) or omega-3 fatty acids to the original AREDS formulation (with beta-carotene) had no overall effect on the risk of late AMD. However, the trial found that replacing beta-carotene with a 5-to-1 mixture of lutein and zeaxanthin could help to further reduce the risk of late AMD, particularly among people who had a low background dietary intake of lutein and zeaxanthin (5,6).

The majority of research performed to date has examined the effects of commonly known antioxidants such as vitamins C, E and A, and carotenoids (lutein and zeaxanthin) on AMD risk and progression. There is limited research on promising phytochemicals with antioxidant and anti-inflammatory properties, including flavonoids (13). Flavonoids are bioactive compounds found in foods such as tea, chocolate, red wine, fruit, and vegetables (14). Flavonoids found in foods can be divided into six main flavonoid classes: flavonols, flavan-3-ols, flavones, flavanones, anthocyanins, and isoflavones (14,15). Flavonoids may have antioxidant and anti-inflammatory activities (15,16). There is also strong evidence that flavonoids positively impact vascular health through improved endothelial function (17). Thus, the role of flavonoids seem promising for reversing oxidative stress and inflammation-associated damage and improving vascular function and thus, possibly improving the clinical features of AMD (13).

However, additional research is needed to establish whether flavonoid intake is beneficially associated with risk of AMD. Hence, we aimed to use a well characterized large cohort of adults aged 49+ years to explore: 1) Associations between dietary intake of total flavonoids with the prevalence and 15-year incidence of AMD (primary endpoints), independent of potential confounders; 2) Prospective relationship between six common flavonoid classes (flavonols, flavan-3-ols, flavones, flavanones, anthocyanins, and isoflavones) and key individual flavonoids (quercetin and hesperidin) with the prevalence and 15-year incidence of AMD; and 3) Associations between the main foods and beverages contributing to total flavonoids e.g. tea, apples, oranges and orange juice, and both the prevalence and 15-year incidence of AMD.

Methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of common eye diseases and other health outcomes in a suburban Australian population located west of Sydney. Study methods and procedures have been described elsewhere (18). Baseline examinations of 3654 residents aged >49 years were conducted during 1992-4 (BMES-1; 82.4% participation rate). Selection bias at baseline was minimised after multiple call-back visits, including door-knocking, telephone reminders and letters at recruitment. Surviving baseline participants were invited to attend examinations after 5- (1997-9, BMES-2), 10- (2002-4, BMES-3), and 15 years (2007-9, BMES-4) at which 2334 (75.1% of survivors), 1952 participants (75.6% of survivors) and 1149 (55.4% of survivors) were re-examined, respectively. Participants who did not return to the 5-year visit were also invited to the 10- or 15-year visits. For the current report we analyzed data from BMES-1 through to BMES-4. The University of Sydney and the Western Sydney Area Human Ethics Committees approved

the study including all methods that were performed, and written informed consent was obtained from all participants at each examination. All methods in this study were performed in accordance with the relevant guidelines and regulations.

Assessment of AMD

We took two 30° stereoscopic color retinal photographs of the macula of both eyes, which were graded for presence of early and late AMD using the Wisconsin AMD Grading System (19,20). Inter- and intra-grader reliability showed good agreement for grading of specific AMD lesions with quadratic weighted kappa values ranging from 0.64 to 0.93 and 0.54-0.94 respectively (21). The detailed methodology of AMD ascertainment in this population has been previously reported (19,20). Early AMD was defined as the absence of late AMD and presence of either: 1) large (>125-µm diameter) indistinct soft or reticular drusen or 2) both large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or hypopigmentation) in either eye (20). Similarly, late AMD was defined as the presence of neovascular AMD or geographic atrophy in either eye (20). Any AMD was defined as having early or late AMD. A retinal specialist (P.M.) adjudicated all uncertain retinal pathology and confirmed all late AMD cases.

Assessment of flavonoid intake

Dietary data were collected using a 145-item self-administered food frequency questionnaire (FFQ). The FFQ is modified for Australian diet and vernacular from an early Willett FFQ (22) and includes reference portion sizes. Participants used a 9-category frequency scale to indicate the usual frequency of consuming individual food items during the past year. Foods listed in the FFQ were categorized into major food categories and subcategories similar to those used for the 1995 Australian National Nutrition Survey (23). Estimates of the flavonoid

content of foods in the FFQ were derived from the US Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods (24), USDA Database for the Isoflavone Content of Selected Foods (25) and USDA Database for the Proanthocyanidin Content of Selected Foods (26).

The method of computing the flavonoid content of foods was similar to that outlined in Mink *et al.* (27) Specifically, for each food, we computed the intake of each individual flavonoid compound present in the food, the sum of assessed flavonoids for each flavonoid class, by summing the individual compounds of each flavonoid class, and the sum of all flavonoid intakes, by summing the flavonoid classes. The flavan-3-ol content of foods was considered to represent the average of total flavan-3-ol and proanthocyanidin monomer contents. For foods where only the flavan-3-ol or proanthocyanidin monomer content was available, the single value provided was used to represent the flavan-3-ol content. The proanthocyanidin content of foods was calculated by summing the proanthocyanidin dimers, trimers, 4–6mers, 7–10mers and polymers. Where multiple varieties of a food listed in the FFQ were reported in the databases, the average flavonoid content of all similar varieties was computed, consistent with the descriptors used in the FFQ output. Foods in the FFQ that were not in the flavonoid databases were assumed to contain no flavonoids. Intakes of flavonoid classes (in mg/d) were calculated by multiplying the estimated intake (g edible portion/d) from the FFQ, with the flavonoid class content (mg/d edible portion) of each food item on the questionnaire. Some of the food items on the FFQ with multiple ingredients (e.g., pizza) were assigned a weighted value on the basis of a USDA standard recipe.

Assessment of covariates

Participants self-reported smoking status as: never smoked; past smoker; or current smoker. We extracted separate data on the frequency of consuming fish (e.g. salmon, tuna and

sardines) and dietary intakes of lutein and zeaxanthin from the FFQ. The United States Department of Agriculture Carotenoid Food Composition database (28) was used to estimate the intakes of other combined lutein and zeaxanthin. Genotypic status was available for the complement factor H (*CFH*) single nucleotide polymorphism (SNP) *rs*1061170 in 2041 baseline participants who returned at BMES2 and for the age-related maculopathy susceptibility gene 2 (*ARMS2*) SNP *rs*10490924 in 1893 baseline participants who returned at BMES2. Two sources of genotypic information were used (29). TaqMan assays (Applied Biosystems, Foster City, CA), had been performed to provide specific genotyping of *rs*1061170 in 1925 individuals and *rs*10490924 in 638 individuals. In addition, BMES genotyping was also carried out for a genome-wide association study (GWAS) using a custom array (Human 670-Quad, version 1, Illumina Inc) at the Wellcome Trust Centre for Human Genetics, Sanger Institute, Cambridge, UK, as part of the Wellcome Trust Case Control Consortium 2. After quality control, genotype imputation was performed using a genetic variation catalogue (1000 Genomes, version 1) and IMPUTE software. Imputed genotypic status was available for *rs*1061170 in 1657 baseline participants who returned at BMES2 and *rs*10490924 in 1802 baseline participants who returned at BMES2. This information on genotyping status from imputed data was used where TaqMan assays were not available, for *rs*1061170 in 116 individuals and for *rs*10490924 in 1255 individuals. Concordance rates between typed and imputed SNP values were 99.6% for *rs*1061170 and 99.2% for *rs*10490924. Imputation data metrics were as follows: imputation R^2 values were 0.968 for *rs*1061170 and 0.996 for *rs*10490924, the proportion of the sample with missing SNP information was 8.8% for *rs*1061170 and 0.5% for *rs*10490924, Hardy Weinberg equilibrium p values were 0.79 for *rs*1061170 and 0.95 for *rs*10490924, minor allele frequencies were 0.39 for *rs*1061170 and 0.22 for *rs*10490924.

Statistical analysis

In exploratory analyses, we assessed associations with the prevalence and 15-year incidence of AMD, which were the primary endpoints. These primary endpoints did not change during the course of the present study or during post-hoc analyses. SAS statistical software (SAS Institute, Cary NC) version 9.4 was used for analyses. Energy-adjusted dietary flavonoid intakes were transformed to normal scores using the Blom method. Associations between energy-adjusted baseline dietary flavonoid intakes (study factor) and prevalence of AMD (study outcome) were examined using logistic regression analysis. Further, associations between energy-adjusted baseline dietary flavonoid intakes and 15-year cumulative incidence of AMD were examined in discrete logistic regression models. The discrete logistic model refers to a survival model in which event times are treated as being genuinely discrete in truth, rather than being on a continuous spectrum. The discrete time hazard is related to covariates by a logistic regression equation (30,31). We have used its implementation in SAS in proc phreg, where a partial likelihood estimation method is used. Findings were also examined after accounting for the competing risk of death using Fine and Gray's model (32) for cumulative incidence in the presence of competing risks. Regression analysis was first adjusted for age and sex, and then for covariates that have been found to be associated with incidence of AMD in the BMES cohort: current smoking, fish consumption, intakes of lutein and zeaxanthin, and the presence of *CFH* and *ARMS2* SNPs, *rs*1061170 and *rs*10490924, respectively. Genotype status was included as an adjustment factor in multivariable-adjusted models using three categories (no minor alleles, one minor allele only, or two minor alleles), based on an additive model for genetic effects. Further adjustments for BMI, hypertension, physical activity (in metabolic equivalents) and dietary vitamin C intake were also considered but did not appreciably change the observed estimates, so were not included in the main

analysis. Findings from all analyses are expressed as adjusted odds ratios (OR) with 95% confidence intervals (CI).

Results

Prevalence of AMD

Of the 3654 subjects examined at baseline, 2856 who had complete dietary data as well as information on AMD lesions were included in the prevalence analysis (**Supplemental Figure 1**). Study characteristics of participants included in cross-sectional analysis are shown in **Table 1**. At baseline, there were 4.6% and 1.7% participants with early and late AMD, respectively (Table 1). After multivariable-adjustment, each 1-SD increase in intake of total flavonoids was associated with reduced likelihood of any AMD, OR 0.76 (95% CI 0.58, 0.99). Each 1-SD increase in intake of total flavonol and total flavanone was associated with reduced odds of any AMD: OR 0.75 (95% CI 0.58, 0.97); and OR 0.77 (95% CI 0.60, 0.99), respectively. Supplementary analysis involved key individual flavonoids - quercetin (a flavonol) and hesperidin (flavanone), and prevalence of AMD. After adjusting for all potential confounders, each 1-SD increase in intake of quercetin was associated with reduced odds of any AMD: OR 0.76 (95% CI 0.58, 0.99). No significant linear associations were observed between hesperidin and prevalence of AMD (data not shown).

Table 2 shows the association between quartiles of intake of flavonoids and prevalence of AMD. Participants in the highest quartile of total flavanone intake compared to those in the lowest quartile of intake had reduced odds of any and early AMD. Those in the highest versus lowest quartile of total flavonol intake had a 57% reduced likelihood of any AMD, after multivariable adjustment. Participants in the highest quartile of total hesperidin intake compared to those in the lowest quartile of intake had reduced odds of any and early AMD (Table 2).

Additional analysis involved investigating the main foods and beverages contributing to total flavonoids, flavonols, and flavanones i.e. apples, oranges, tea and orange juice. Compared to participants who did not consume any oranges (reference group), those who reported having one or more serves of oranges per week but less than one serve per day had reduced odds of any AMD: multivariable-adjusted OR 0.42 (95% CI 0.21, 0.84). Similarly, participants who reported one or more serves of oranges per day compared to the reference group had reduced odds of any AMD OR 0.42 (95% CI 0.20, 0.89). Also, compared to participants who did not consume any oranges, participants who ate one or more serves of oranges per week but had less than one serve per day had 92% reduced odds of late AMD: OR 0.08 (95% CI 0.01, 0.76). Participants who consumed one or more serves of orange juice per day compared to those who never consumed orange juice had reduced likelihood of having early AMD: multivariable-adjusted OR 0.35 (95% CI 0.14, 0.85). No significant associations were observed between consumption of apples, tea, red wine and beer with prevalence of AMD (data not shown).

Incidence of AMD

Of the 2856 included in the prevalence analysis, 2037 participants with complete AMD and lifestyle data were re-examined 5, 10 and/or 15 years later (i.e. at least one follow-up examination), and therefore included in incidence analysis (Supplemental Figure 1). Baseline characteristics of participants included in longitudinal analysis are shown in Table 1. There were 15.3% and 4.1% incident early and late AMD cases, respectively. No significant linear associations were observed between flavonoid intake and 15-year incidence of AMD (data not shown). A marginally significant trend was observed between increasing intake of total hesperidin (from first to fourth quartile) and lower 15-year incidence of late AMD, after multivariable adjustment (**Table 3**). Findings were essentially similar after accounting for the

competing risk of death, except that the trend across quartiles of hesperidin became marginally non-significant (p=0.06), while a significant trend emerged between quartiles of increasing flavonol intake and increased incidence of early AMD (p=0.03). Participants who reported one or more serves of oranges per day versus those who never ate oranges at baseline had reduced risk of incident late AMD 15 years later: multivariable-adjusted OR 0.39 (95% CI 0.18, 0.85). No significant associations were observed between consumption of apples, orange juice, tea, red wine and beer with the 15-year incidence of AMD (data not shown).

Discussion

This prospective cohort study of older adults provides novel epidemiological evidence of an independent association between total flavonoid intake as well as the intake of specific flavonoid classes and AMD. Specifically, we observed significant and protective associations between the intake of total flavonoids as well as total flavonol and total flavanone intake with AMD prevalence. Modest associations were also observed between the intakes of total flavone, flavanone and hesperidin and risk of incident late AMD 15 years later. Our study suggests that consumption of oranges (a key contributor to total flavanone) is inversely and independently associated with both prevalence and incidence of late AMD.

The median intake of total flavonoids in our cohort was 875 mg/day which is higher than that previously reported in a Western Australia cohort (median intake of 696-mg/d in women aged >75 years) (14) and in an Australia-wide nutrition survey (median intake of 454 mg/day in those aged 19+years) (33). This difference is likely to be due to variations in age-distribution, However, variations in food content databases and the different dietary assessment methods administered could also explain the differences in flavonoid intake observed between studies (14).

Higher total overall flavonoid intake and intake of particular flavonoid subgroups e.g. flavonol and flavanone, were associated with reduced odds of having AMD. This observed association is in line with existing evidence, as flavonoids are found in abundance in fruits and vegetables (15) and adequate consumption of fruits and vegetables has been established as being protective against AMD (2,34). Our findings also concur with the existing published literature which has shown that following consumption, flavonoids may contribute to a variety of beneficial biological activities in humans (14). There is robust data now showing that flavonoids can preserve and enhance nitric oxide status and improve endothelial function (35,36). There is also evidence that these compounds can minimize oxidative damage and inflammation (15,16). Moreover, among the known angiogenesis inhibitors, flavonoids seem to play an important role (37). While, the mechanism behind the antiangiogenic effect of flavonoids is unclear, one proposed pathway is through inhibition of protein kinases (2). Overall, these salutary effects of flavonoids may help to explain the influence these dietary compounds might have on AMD pathogenic processes, that is, the inflammatory, oxidative and angiogenic pathways (38).

The associations between flavonoid intake and both AMD prevalence and incidence appear to be class dependent. Specifically, participants with higher intakes of flavonol and flavanone had reduced odds of any AMD, while other flavonoid classes such as flavan-3-ols and isoflavone did not show any significant associations with AMD prevalence. Similarly, differential associations with 15-year incidence of AMD were observed e.g. flavone and flavanone intakes were inversely associated with risk of incident AMD while other flavonoid subgroups were not. The varying structures and bioactivities of the different flavonoid classes, as well as the ability to adequately assess intakes from foods could explain the differential associations observed between the individual flavonoid classes and AMD prevalence and incidence (14,39). Indeed, even a minor structural difference in flavonoids

can have a large impact on their bioavailability (40,41). Further studies are needed to confirm our findings and elucidate the influence of total flavonoids and flavonoid subclasses on the development and progression of AMD in older adults.

Our findings are promising, as BMES data show for the first time that flavonoids may be useful food compounds in protecting against AMD. Oral bioavailability of flavonoids, however, is known to be limited by poor intrinsic transmembrane diffusion characteristics and poor solubility (42). Moreover, the activity of the flavonoid metabolites is not well established (42). Further research is also needed to establish whether systemic administration of flavonoids will yield much higher and effective concentrations of the parent flavonoids in the ocular tissues and at much lower doses (42). For the time being, it is reasonable that adequate intake of fruits (particularly oranges), vegetables, and beverages (e.g. orange juice) containing flavonoids be recommended to patients, although it is too early to make recommendations on daily flavonoid intakes for prevention of AMD (15). Strengths of this study include its prospective data collection, long-term follow-up of a population-based sample, use of a validated FFQ and careful adjustment for confounders including genetic risk. Hence, our findings are applicable to the general older Australian population and could also be applicable to older adults in other Western countries. Additionally, this study uses high quality stereoscopic retinal photography with validated grading to assess macular conditions, and a detailed side-by-side comparison of the baseline and follow-up photographs to ensure negligible misclassification of incident AMD (4,43,44). However, this study has some noteworthy limitations. First, the database used for the estimation of flavonoid content of foods is based on US data only and therefore this approach might not have accounted for any variation in the flavonoid content of foods found in Australia (40). Second, we cannot discount the effect of residual confounding from unmeasured or unaccounted factors (e.g. inflammatory markers) on observed associations. Finally, the number of participants who

306 developed incident AMD was small, and this might have reduced power to detect modest
307 associations with flavonoid intake.
308 In summary, we report novel independent associations between dietary intake of total
309 flavonoids, and some of the common flavonoid classes (e.g. flavonol and flavanone) and
310 AMD among older adults. Further, oranges and orange juice, one of the main foods and
311 beverages contributing to total flavanone, is also likely to independently influence risk of
312 AMD. These findings suggest that a habitual diet high in flavonoids could play a role in
313 AMD prevention and progression. These associations, if confirmed in other epidemiological
314 and intervention studies could have important public health implications.

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Conflicts of interest statement

None to declare.

Authors' Contributions

BG and PM - designed research; PM, BG, VMF, JH and JL - conducted research; AK - analyzed data or performed statistical analysis; BG, PM, GL - wrote paper; and BG - had primary responsibility for final content.

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Table 1. Baseline characteristics of participants involved in prevalence and 15-year incidence age-related macular degeneration (AMD) analysis

Characteristics	**Prevalence (n=2856)**	**Incidence (n=2037)**
Age, *yrs*	65.3 (9.3)	63.8 (8.3)
Males	1259 (44.1)	881 (43.3)
Current smokers	393 (14.2)	247 (12.4)
Fish consumption (≥1 serve/ week)	1690 (59.8)	1200 (59.4)
Presence of one or two AMD risk alleles		
CFH-rs1061170	1077 (60.9)	1051 (60.6)
ARMS2-rs10490924	629 (38.4)	607 (37.9)
AMD type		
Early	130 (4.6)	268 (15.3)
Late	47 (1.7)	84 (4.1)

Data is presented as mean (±SD) or n (%) and *p*-values were obtained using *t*-tests for continuous variables and chi-square analyses for categorical data.

Table 2. Associations between flavonoid intake and prevalence of age-related macular degeneration (AMD) in the Blue Mountains Eye Study (n=2856)

Flavonoids (mg/day)	Any AMD (n=177)	Early AMD (n=130)	Late AMD (n=47)
	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)
All flavonoids[1]			
1st quartile (≤410.6)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd quartile (412.4-881.5)	0.63 (0.31, 1.29)	0.51 (0.23, 1.14)	1.37 (0.31, 6.13)
3rd quartile (881.6-1232.3)	0.62 (0.31, 1.24)	0.63 (0.30, 1.32)	0.60 (0.11, 3.10)
4th quartile (≥1232.4)	0.52 (0.25, 1.06)	0.52 (0.24, 1.12)	0.45 (0.08, 2.60)
P for trend	0.08	0.12	0.25
Total flavonol[1]			
1st quartile (≤18.2)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd quartile (18.3-34.6)	**0.43 (0.20, 0.90)**	0.46 (0.20, 1.07)	0.28 (0.06, 1.28)
3rd quartile (34.6-46.0)	0.70 (0.36, 1.36)	0.82 (0.40, 1.69)	0.33 (0.08, 1.38)
4th quartile (≥46.0)	**0.43 (0.21, 0.88)**	0.48 (0.22, 1.07)	0.22 (0.04, 1.02)
P for trend	**0.05**	0.16	**0.05**
Total flavanone[1]			
1st quartile (≤9.6)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd quartile (9.6-25.1)	0.51 (0.24, 1.06)	**0.34 (0.14, 0.83)**	1.61 (0.40, 6.50)
3rd quartile (25.2-47.1)	1.01 (0.55, 1.85)	1.05 (0.55, 1.99)	0.84 (0.18, 3.94)
4th quartile (≥47.2)	**0.29 (0.13, 0.66)**	**0.25 (0.10, 0.63)**	0.65 (0.10, 4.06)
P for trend	**0.01**	**0.02**	0.46
Total quercetin			
1st quartile (≤12.3)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd quartile (12.3-20.8)	**0.46 (0.22, 0.99)**	0.52 (0.23, 1.18)	0.26 (0.05, 1.48)
3rd quartile (20.8-26.9)	0.73 (0.37, 1.43)	0.76 (0.36, 1.60)	0.65 (0.17, 2.52)
4th quartile (≥26.9)	0.49 (0.24, 1.00)	0.53 (0.24, 1.17)	0.27 (0.05, 1.39)
P for trend	0.12	0.21	0.20
Total hesperidin			
1st quartile (≤5.5)	1.0 (reference)	1.0 (reference)	1.0 (reference)

2nd quartile (5.5-16.0)	0.63 (0.31, 1.26)	0.49 (0.22, 1.09)	1.44 (0.36, 5.85)
3rd quartile (16.0-30.1)	0.76 (0.40, 1.46)	0.78 (0.39, 1.54)	0.76 (0.15, 3.96)
4th quartile (≥30.2)	**0.47 (0.23, 0.97)**	**0.43 (0.19, 0.93)**	0.92 (0.18, 4.74)
P for trend	0.08	0.10	0.70

OR – odds ratio; CI – confidence intervals. Bolded values represent significant associations ($p < 0.05$) in comparison to the reference group.

[1] Values were calculated by using logistic regression analyses and were adjusted for age, sex, current smoking, fish consumption, intakes of lutein and zeaxanthin, and *CFH* and *ARMS2* SNPS (*rs1061170* and *rs10490924*).

Table 3. Associations between flavonoid intake and 15-year incidence of age-related macular degeneration (AMD) in the Blue Mountains Eye Study (n=2037)

Flavonoids (mg/day)	Early AMD (n=268)	Late AMD (n=84)
	Adjusted OR (95% CI)	Adjusted OR (95% CI)
All flavonoids[1]		
1st quartile (≤410.1)	1.0 (reference)	1.0 (reference)
2nd quartile (413.0-881.5)	1.13 (0.75, 1.71)	0.72 (0.33, 1.58)
3rd quartile (882.0-1232.3)	0.94 (0.62, 1.42)	1.17 (0.60, 2.29)
4th quartile (≥1232.4)	1.22 (0.82, 1.81)	1.00 (0.50, 2.00)
P for trend	0.35	0.65
Total flavone[1]		
1st quartile (≤0.64)	1.0 (reference)	1.0 (reference)
2nd quartile (0.7-1.0)	0.97 (0.66, 1.44)	**2.36 (1.13, 5.01)**
3rd quartile (1.0-1.5)	0.83 (0.56, 1.23)	1.46 (0.66, 3.23)
4th quartile (≥1.5)	0.75 (0.50, 1.11)	1.52 (0.66, 3.49)
P for trend	0.10	0.97
Total flavanone[1]		
1st quartile (≤9.6)	1.0 (reference)	1.0 (reference)
2nd quartile (9.6-25.1)	0.92 (0.62, 1.38)	1.15 (0.62, 2.11)
3rd quartile (25.2-47.1)	0.97 (0.67, 1.41)	0.69 (0.36, 1.32)
4th quartile (≥47.2)	0.82 (0.55, 1.22)	0.55 (0.27, 1.09)
P for trend	0.30	0.05
Total hesperidin[1]		
1st quartile (≤5.5)	1.0 (reference)	1.0 (reference)
2nd quartile (5.5-16.0)	1.03 (0.69, 1.53)	1.22 (0.65, 2.27)
3rd quartile (16.0-30.1)	1.11 (0.76, 1.62)	0.88 (0.46, 1.68)
4th quartile (≥30.2)	0.85 (0.57, 1.26)	0.54 (0.26, 1.13)
P for trend	0.32	**0.05**

OR – odds ratio; CI – confidence intervals. Bolded values represent significant associations ($p < 0.05$) in comparison to the reference group.

[1] Values were calculated by using discrete logistic regression models and were adjusted for age, sex, current smoking, fish consumption, intakes of lutein and zeaxanthin, and *CFH* and *ARMS2* SNPS (*rs1061170* and *rs10490924*).