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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

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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

1 **Abstract**

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

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

10 **Design:** In this population-based cohort study, 2856 adults aged 49+ years at baseline and
11 2037 followed up 15-years later were included in prevalence and incidence analysis,
12 respectively. Dietary intake was assessed using a semi-quantitative food-frequency
13 questionnaire (FFQ). Estimates of the flavonoid content of foods in the FFQ were assessed
14 using the US Department of Agriculture Flavonoid, Isoflavone and Proanthocyanidin
15 databases. AMD was assessed from retinal photographs.

16 **Results:** In cross-sectional analysis, each 1-SD increase in total overall flavonoid intake was
17 associated with reduced likelihood of any AMD, multivariable-adjusted OR 0.76 (95% CI
18 0.58, 0.99). Each 1-SD increase in dietary intake of total flavonol and total flavanone was
19 associated with reduced odds of prevalent any AMD: multivariable-adjusted OR 0.75 (95%
20 CI 0.58, 0.97); and OR 0.77 (95% CI 0.60, 0.99), respectively. A marginally significant trend
21 ($p=0.05$) was observed between increasing intake of total flavanone and hesperidin (from first
22 to fourth quartile) and reduced likelihood of incident late AMD, after multivariable
23 adjustment. Participants who reported one or more serves of oranges per day versus those
24 who never ate oranges at baseline had reduced risk of late AMD 15 years later: multivariable-
25 adjusted OR 0.39 (95% CI 0.18, 0.85).

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

29

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

32 **Introduction**

33 Age-related macular degeneration (AMD) is the leading cause of blindness and severe visual
34 impairment in older adults (1). Current evidence suggests that AMD patients should be given
35 dietary advice to increase consumption of dark green leafy vegetables, to consume low-
36 glycemic index diets, and to consume fish at least twice a week (2-6). The Age-Related Eye
37 Disease Study (AREDS) demonstrated that taking a supplement containing high doses of
38 vitamin C, vitamin E, beta-carotene, zinc, and copper could reduce AMD progression by 25%
39 (7-12). A follow-up study (AREDS 2), found adding lutein and zeaxanthin (naturally
40 occurring carotenoids) or omega-3 fatty acids to the original AREDS formulation (with beta-
41 carotene) had no overall effect on the risk of late AMD. However, the trial found that
42 replacing beta-carotene with a 5-to-1 mixture of lutein and zeaxanthin could help to further
43 reduce the risk of late AMD, particularly among people who had a low background dietary
44 intake of lutein and zeaxanthin (5,6).

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

57 However, additional research is needed to establish whether flavonoid intake is
58 beneficially associated with risk of AMD. Hence, we aimed to use a well characterized large
59 cohort of adults aged 49+ years to explore: 1) Associations between dietary intake of total
60 flavonoids with the prevalence and 15-year incidence of AMD (primary endpoints),
61 independent of potential confounders; 2) Prospective relationship between six common
62 flavonoid classes (flavonols, flavan-3-ols, flavones, flavanones, anthocyanins, and
63 isoflavones) and key individual flavonoids (quercetin and hesperidin) with the prevalence and
64 15-year incidence of AMD; and 3) Associations between the main foods and beverages
65 contributing to total flavonoids e.g. tea, apples, oranges and orange juice, and both the
66 prevalence and 15-year incidence of AMD.

67

68 **Methods**

69 *Study population*

70 The Blue Mountains Eye Study (BMES) is a population-based cohort study of common eye
71 diseases and other health outcomes in a suburban Australian population located west of
72 Sydney. Study methods and procedures have been described elsewhere (18). Baseline
73 examinations of 3654 residents aged >49 years were conducted during 1992-4 (BMES-1;
74 82.4% participation rate). Selection bias at baseline was minimised after multiple call-back
75 visits, including door-knocking, telephone reminders and letters at recruitment. Surviving
76 baseline participants were invited to attend examinations after 5- (1997-9, BMES-2), 10-
77 (2002-4, BMES-3), and 15 years (2007-9, BMES-4) at which 2334 (75.1% of survivors),
78 1952 participants (75.6% of survivors) and 1149 (55.4% of survivors) were re-examined,
79 respectively. Participants who did not return to the 5-year visit were also invited to the 10- or
80 15-year visits. For the current report we analyzed data from BMES-1 through to BMES-4.
81 The University of Sydney and the Western Sydney Area Human Ethics Committees approved

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

85

86 *Assessment of AMD*

87 We took two 30° stereoscopic color retinal photographs of the macula of both eyes, which
88 were graded for presence of early and late AMD using the Wisconsin AMD Grading System
89 (19,20). Inter- and intra-grader reliability showed good agreement for grading of specific
90 AMD lesions with quadratic weighted kappa values ranging from 0.64 to 0.93 and 0.54-0.94
91 respectively (21). The detailed methodology of AMD ascertainment in this population has
92 been previously reported (19,20). Early AMD was defined as the absence of late AMD and
93 presence of either: 1) large (>125- μ m diameter) indistinct soft or reticular drusen or 2) both
94 large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or
95 hypopigmentation) in either eye (20). Similarly, late AMD was defined as the presence of
96 neovascular AMD or geographic atrophy in either eye (20). Any AMD was defined as having
97 early or late AMD. A retinal specialist (P.M.) adjudicated all uncertain retinal pathology and
98 confirmed all late AMD cases.

99

100 *Assessment of flavonoid intake*

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

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

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

128

129 *Assessment of covariates*

130 Participants self-reported smoking status as: never smoked; past smoker; or current smoker.

131 We extracted separate data on the frequency of consuming fish (e.g. salmon, tuna and

132 sardines) and dietary intakes of lutein and zeaxanthin from the FFQ. The United States
133 Department of Agriculture Carotenoid Food Composition database (28) was used to estimate
134 the intakes of other combined lutein and zeaxanthin. Genotypic status was available for the
135 complement factor H (*CFH*) single nucleotide polymorphism (SNP) *rs1061170* in 2041
136 baseline participants who returned at BMES2 and for the age-related maculopathy
137 susceptibility gene 2 (*ARMS2*) SNP *rs10490924* in 1893 baseline participants who returned at
138 BMES2. Two sources of genotypic information were used (29). TaqMan assays (Applied
139 Biosystems, Foster City, CA), had been performed to provide specific genotyping of
140 *rs1061170* in 1925 individuals and *rs10490924* in 638 individuals. In addition, BMES
141 genotyping was also carried out for a genome-wide association study (GWAS) using a
142 custom array (Human 670-Quad, version 1, Illumina Inc) at the Wellcome Trust Centre for
143 Human Genetics, Sanger Institute, Cambridge, UK, as part of the Wellcome Trust Case
144 Control Consortium 2. After quality control, genotype imputation was performed using a
145 genetic variation catalogue (1000 Genomes, version 1) and IMPUTE software. Imputed
146 genotypic status was available for *rs1061170* in 1657 baseline participants who returned at
147 BMES2 and *rs10490924* in 1802 baseline participants who returned at BMES2. This
148 information on genotyping status from imputed data was used where TaqMan assays were
149 not available, for *rs1061170* in 116 individuals and for *rs10490924* in 1255 individuals.
150 Concordance rates between typed and imputed SNP values were 99.6% for *rs1061170* and
151 99.2% for *rs10490924*. Imputation data metrics were as follows: imputation R^2 values were
152 0.968 for *rs1061170* and 0.996 for *rs10490924*, the proportion of the sample with missing
153 SNP information was 8.8% for *rs1061170* and 0.5% for *rs10490924*, Hardy Weinberg
154 equilibrium p values were 0.79 for *rs1061170* and 0.95 for *rs10490924*, minor allele
155 frequencies were 0.39 for *rs1061170* and 0.22 for *rs10490924*.

156

157 *Statistical analysis*

158 In exploratory analyses, we assessed associations with the prevalence and 15-year incidence
159 of AMD, which were the primary endpoints. These primary endpoints did not change during
160 the course of the present study or during post-hoc analyses. SAS statistical software (SAS
161 Institute, Cary NC) version 9.4 was used for analyses. Energy-adjusted dietary flavonoid
162 intakes were transformed to normal scores using the Blom method. Associations between
163 energy-adjusted baseline dietary flavonoid intakes (study factor) and prevalence of AMD
164 (study outcome) were examined using logistic regression analysis. Further, associations
165 between energy-adjusted baseline dietary flavonoid intakes and 15-year cumulative incidence
166 of AMD were examined in discrete logistic regression models. The discrete logistic model
167 refers to a survival model in which event times are treated as being genuinely discrete in
168 truth, rather than being on a continuous spectrum. The discrete time hazard is related to
169 covariates by a logistic regression equation (30,31). We have used its implementation in SAS
170 in proc phreg, where a partial likelihood estimation method is used. Findings were also
171 examined after accounting for the competing risk of death using Fine and Gray's model (32)
172 for cumulative incidence in the presence of competing risks. Regression analysis was first
173 adjusted for age and sex, and then for covariates that have been found to be associated with
174 incidence of AMD in the BMES cohort: current smoking, fish consumption, intakes of lutein
175 and zeaxanthin, and the presence of *CFH* and *ARMS2* SNPs, *rs1061170* and *rs10490924*,
176 respectively. Genotype status was included as an adjustment factor in multivariable-adjusted
177 models using three categories (no minor alleles, one minor allele only, or two minor alleles),
178 based on an additive model for genetic effects. Further adjustments for BMI, hypertension,
179 physical activity (in metabolic equivalents) and dietary vitamin C intake were also considered
180 but did not appreciably change the observed estimates, so were not included in the main

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

183

184 **Results**

185 *Prevalence of AMD*

186 Of the 3654 subjects examined at baseline, 2856 who had complete dietary data as well as
187 information on AMD lesions were included in the prevalence analysis (**Supplemental Figure**
188 **1**). Study characteristics of participants included in cross-sectional analysis are shown in
189 **Table 1**. At baseline, there were 4.6% and 1.7% participants with early and late AMD,
190 respectively (Table 1). After multivariable-adjustment, each 1-SD increase in intake of total
191 flavonoids was associated with reduced likelihood of any AMD, OR 0.76 (95% CI 0.58,
192 0.99). Each 1-SD increase in intake of total flavonol and total flavanone was associated with
193 reduced odds of any AMD: OR 0.75 (95% CI 0.58, 0.97); and OR 0.77 (95% CI 0.60, 0.99),
194 respectively. Supplementary analysis involved key individual flavonoids - quercetin (a
195 flavonol) and hesperidin (flavanone), and prevalence of AMD. After adjusting for all
196 potential confounders, each 1-SD increase in intake of quercetin was associated with reduced
197 odds of any AMD: OR 0.76 (95% CI 0.58, 0.99). No significant linear associations were
198 observed between hesperidin and prevalence of AMD (data not shown).

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

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

220

221 *Incidence of AMD*

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

231 competing risk of death, except that the trend across quartiles of hesperidin became
232 marginally non-significant ($p=0.06$), while a significant trend emerged between quartiles of
233 increasing flavonol intake and increased incidence of early AMD ($p=0.03$). Participants who
234 reported one or more serves of oranges per day versus those who never ate oranges at
235 baseline had reduced risk of incident late AMD 15 years later: multivariable-adjusted OR
236 0.39 (95% CI 0.18, 0.85). No significant associations were observed between consumption of
237 apples, orange juice, tea, red wine and beer with the 15-year incidence of AMD (data not
238 shown).

239

240 **Discussion**

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

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

256 Higher total overall flavonoid intake and intake of particular flavonoid subgroups e.g.
257 flavonol and flavanone, were associated with reduced odds of having AMD. This observed
258 association is in line with existing evidence, as flavonoids are found in abundance in fruits
259 and vegetables (15) and adequate consumption of fruits and vegetables has been established
260 as being protective against AMD (2,34). Our findings also concur with the existing published
261 literature which has shown that following consumption, flavonoids may contribute to a
262 variety of beneficial biological activities in humans (14). There is robust data now showing
263 that flavonoids can preserve and enhance nitric oxide status and improve endothelial function
264 (35,36). There is also evidence that these compounds can minimize oxidative damage and
265 inflammation (15,16). Moreover, among the known angiogenesis inhibitors, flavonoids seem
266 to play an important role (37). While, the mechanism behind the antiangiogenic effect of
267 flavonoids is unclear, one proposed pathway is through inhibition of protein kinases (2).
268 Overall, these salutary effects of flavonoids may help to explain the influence these dietary
269 compounds might have on AMD pathogenic processes, that is, the inflammatory, oxidative
270 and angiogenic pathways (38).

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

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

284 Our findings are promising, as BMES data show for the first time that flavonoids may
285 be useful food compounds in protecting against AMD. Oral bioavailability of flavonoids,
286 however, is known to be limited by poor intrinsic transmembrane diffusion characteristics
287 and poor solubility (42). Moreover, the activity of the flavonoid metabolites is not well
288 established (42). Further research is also needed to establish whether systemic administration
289 of flavonoids will yield much higher and effective concentrations of the parent flavonoids in
290 the ocular tissues and at much lower doses (42). For the time being, it is reasonable that
291 adequate intake of fruits (particularly oranges), vegetables, and beverages (e.g. orange juice)
292 containing flavonoids be recommended to patients, although it is too early to make
293 recommendations on daily flavonoid intakes for prevention of AMD (15). Strengths of this
294 study include its prospective data collection, long-term follow-up of a population-based
295 sample, use of a validated FFQ and careful adjustment for confounders including genetic risk.
296 Hence, our findings are applicable to the general older Australian population and could also
297 be applicable to older adults in other Western countries. Additionally, this study uses high
298 quality stereoscopic retinal photography with validated grading to assess macular conditions,
299 and a detailed side-by-side comparison of the baseline and follow-up photographs to ensure
300 negligible misclassification of incident AMD (4,43,44). However, this study has some
301 noteworthy limitations. First, the database used for the estimation of flavonoid content of
302 foods is based on US data only and therefore this approach might not have accounted for any
303 variation in the flavonoid content of foods found in Australia (40). Second, we cannot
304 discount the effect of residual confounding from unmeasured or unaccounted factors (e.g.
305 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		
<i>CFH-rs1061170</i>	1077 (60.9)	1051 (60.6)
<i>ARMS2-rs10490924</i>	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 (\pm 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 flavonoids¹			
1 st quartile (≤ 410.6)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2 nd quartile (412.4-881.5)	0.63 (0.31, 1.29)	0.51 (0.23, 1.14)	1.37 (0.31, 6.13)
3 rd quartile (881.6-1232.3)	0.62 (0.31, 1.24)	0.63 (0.30, 1.32)	0.60 (0.11, 3.10)
4 th quartile (≥ 1232.4)	0.52 (0.25, 1.06)	0.52 (0.24, 1.12)	0.45 (0.08, 2.60)
<i>P</i> for trend	0.08	0.12	0.25
Total flavonol¹			
1 st quartile (≤ 18.2)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2 nd quartile (18.3-34.6)	0.43 (0.20, 0.90)	0.46 (0.20, 1.07)	0.28 (0.06, 1.28)
3 rd quartile (34.6-46.0)	0.70 (0.36, 1.36)	0.82 (0.40, 1.69)	0.33 (0.08, 1.38)
4 th quartile (≥ 46.0)	0.43 (0.21, 0.88)	0.48 (0.22, 1.07)	0.22 (0.04, 1.02)
<i>P</i> for trend	0.05	0.16	0.05
Total flavanone¹			
1 st quartile (≤ 9.6)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2 nd quartile (9.6-25.1)	0.51 (0.24, 1.06)	0.34 (0.14, 0.83)	1.61 (0.40, 6.50)
3 rd quartile (25.2-47.1)	1.01 (0.55, 1.85)	1.05 (0.55, 1.99)	0.84 (0.18, 3.94)
4 th quartile (≥ 47.2)	0.29 (0.13, 0.66)	0.25 (0.10, 0.63)	0.65 (0.10, 4.06)
<i>P</i> for trend	0.01	0.02	0.46
Total quercetin			
1 st quartile (≤ 12.3)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2 nd quartile (12.3-20.8)	0.46 (0.22, 0.99)	0.52 (0.23, 1.18)	0.26 (0.05, 1.48)
3 rd quartile (20.8-26.9)	0.73 (0.37, 1.43)	0.76 (0.36, 1.60)	0.65 (0.17, 2.52)
4 th quartile (≥ 26.9)	0.49 (0.24, 1.00)	0.53 (0.24, 1.17)	0.27 (0.05, 1.39)
<i>P</i> for trend	0.12	0.21	0.20
Total hesperidin			
1 st quartile (≤ 5.5)	1.0 (reference)	1.0 (reference)	1.0 (reference)

2 nd quartile (5.5-16.0)	0.63 (0.31, 1.26)	0.49 (0.22, 1.09)	1.44 (0.36, 5.85)
3 rd quartile (16.0-30.1)	0.76 (0.40, 1.46)	0.78 (0.39, 1.54)	0.76 (0.15, 3.96)
4 th quartile (\geq 30.2)	0.47 (0.23, 0.97)	0.43 (0.19, 0.93)	0.92 (0.18, 4.74)
<i>P</i> 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 flavonoids¹		
1 st quartile (≤ 410.1)	1.0 (reference)	1.0 (reference)
2 nd quartile (413.0-881.5)	1.13 (0.75, 1.71)	0.72 (0.33, 1.58)
3 rd quartile (882.0-1232.3)	0.94 (0.62, 1.42)	1.17 (0.60, 2.29)
4 th quartile (≥ 1232.4)	1.22 (0.82, 1.81)	1.00 (0.50, 2.00)
<i>P</i> for trend	0.35	0.65
Total flavone¹		
1 st quartile (≤ 0.64)	1.0 (reference)	1.0 (reference)
2 nd quartile (0.7-1.0)	0.97 (0.66, 1.44)	2.36 (1.13, 5.01)
3 rd quartile (1.0-1.5)	0.83 (0.56, 1.23)	1.46 (0.66, 3.23)
4 th quartile (≥ 1.5)	0.75 (0.50, 1.11)	1.52 (0.66, 3.49)
<i>P</i> for trend	0.10	0.97
Total flavanone¹		
1 st quartile (≤ 9.6)	1.0 (reference)	1.0 (reference)
2 nd quartile (9.6-25.1)	0.92 (0.62, 1.38)	1.15 (0.62, 2.11)
3 rd quartile (25.2-47.1)	0.97 (0.67, 1.41)	0.69 (0.36, 1.32)
4 th quartile (≥ 47.2)	0.82 (0.55, 1.22)	0.55 (0.27, 1.09)
<i>P</i> for trend	0.30	0.05
Total hesperidin¹		
1 st quartile (≤ 5.5)	1.0 (reference)	1.0 (reference)
2 nd quartile (5.5-16.0)	1.03 (0.69, 1.53)	1.22 (0.65, 2.27)
3 rd quartile (16.0-30.1)	1.11 (0.76, 1.62)	0.88 (0.46, 1.68)
4 th quartile (≥ 30.2)	0.85 (0.57, 1.26)	0.54 (0.26, 1.13)
<i>P</i> 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*).