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1 **Associations between dietary flavonoids and retinal microvasculature in older adults**

2 *Running title:* Flavonoids and retinal vessels

3

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29

30 Abbreviations:

31 BMES - Blue Mountains Eye Study

32 CRAE - Central Retinal Artery Equivalent

33 CRVE - Central Retinal Vein Equivalent

34 CVD - Cardiovascular Disease

35 FFQ - Food-Frequency Questionnaire

36 GLM - General Linear Model

37 USDA - US Department of Agriculture

38

39 **Abstract**

40 **Purpose:** In this study, we assessed whether there are independent associations between dietary
41 total flavonoid intake and major flavonoid classes with retinal arteriolar and venular calibre.

42 **Methods:** Blue Mountains Eye Study participants aged 49+ years who had complete data on diet
43 and retinal vessel measures were analyzed (n=2821). Dietary intake was assessed using a semi-
44 quantitative food-frequency questionnaire (FFQ). Flavonoid content of foods in the FFQ was
45 estimated using the US Department of Agriculture Flavonoid, Isoflavone and Proanthocyanidin
46 databases. Fundus photographs were taken and retinal vascular calibre was measured using
47 validated computer-assisted techniques. The associations of intake of dietary flavonoids with retinal
48 vessel calibre was examined in linear regression models and general linear model.

49 **Results:** The highest quartile of intake was compared with the lowest quartile using multivariable
50 adjustment models. Participants with the highest proanthocyanidin intake had narrower retinal
51 venules (223.9 ± 0.62 versus 226.5 ± 0.63 , respectively; $P_{\text{trend}}=0.01$); and the highest isoflavone intake
52 was associated with wider retinal arterioles (188.1 ± 0.55 versus 186.3 ± 0.56 , respectively;
53 $P_{\text{trend}}=0.01$). The highest apple/pear consumption (a dietary source of catechin) was associated with
54 narrower retinal venules (223.8 ± 0.57 versus 226.1 ± 0.52 ; $P_{\text{trend}}=0.01$) and wider retinal arterioles
55 (187.9 ± 0.51 versus 186.2 ± 0.51 ; $P_{\text{trend}}=0.02$). Further, participants who were in the highest versus
56 lowest quartile of chocolate consumption had $\sim 2.1 \mu\text{m}$ narrower retinal venules (multivariable-
57 adjusted $p=0.03$).

58 **Conclusions:** This study shows that higher intakes of specific flavonoid subclasses are associated
59 with a favourable retinal microvascular profile. Greater consumption of flavonoid-rich apples/pears
60 and chocolate was also associated with beneficial variations in retinal vascular calibre.

61

62 **Keywords:** Blue Mountains Eye Study; flavonoids; retinal vascular calibre.

63

64 **Introduction**

65 There is increasing evidence that measures of retinal microvascular health are associated with early
66 stages of subclinical cardiovascular disease (CVD) and are predictive of clinical CVD events [1-3].

67 The retinal microvasculature may provide an integrated assessment of the microvascular
68 consequences of exposure to vascular insults and may be a valuable prognostic tool in predicting
69 future CVD risk [1,2,4]. A beneficial retinal microvascular profile is typically characterized by
70 wider retinal arterioles and narrower retinal venules [1,2,4]. We previously showed that individual
71 dietary factors associated with CVD risk factors and related events, such as dairy foods,
72 carbohydrates and overall diet quality, were also independently related to characteristics of the
73 retinal microvasculature [5-7].

74 Flavonoids are bioactive compounds found in plant-based foods and beverages such as tea,
75 chocolate, red wine, fruit, and vegetables [8]. Recent studies indicate that certain dietary flavonoids
76 positively impact on cardiovascular health via effects on nitric oxide (NO) bioavailability,
77 endothelial function, blood pressure and chronic inflammation [9-11]. Given that adverse retinal
78 vascular calibre changes are proposed to reflect inflammation, endothelial dysfunction, and
79 hypertension [4,12]; the beneficial role of dietary flavonoids may be partly mediated through their
80 influence on the microvasculature. Further, flavonoids could influence the microvasculature via
81 vascular endothelial growth factor (VEGF). VEGF has a significant role in the normal retina [13],
82 for example, VEGF induces vessel dilation and hence, increases ocular blood flow via a mechanism
83 involving nitric oxide [14]. There is data to suggest that flavonoid compounds e.g. quercetin, inhibit
84 vascular endothelial growth factor (VEGF)-induced choroidal and retinal angiogenesis in vitro [15].
85 Moreover, flavonoid compounds have shown to protect against ocular conditions. Specifically,
86 intake of anthocyanins was shown to be associated with vision improvement and quercetin intake
87 was shown to protect against hydrogen peroxide-induced cataracts and diabetes-induced retinal
88 lesions [16]. Our group also recently showed that intake of hesperidin protected against the
89 development of late-stage age-related macular degeneration [17].

90 To our best knowledge, no prior epidemiological study has investigated the association
91 between dietary flavonoids and retinal microvascular structure. Therefore, using a large
92 representative cohort of adults aged 49+ years, we aimed to assess the cross-sectional associations
93 between flavonoid intakes and retinal arteriolar and venular calibre. Associations were explored for
94 total flavonoids and major flavonoid subclasses including flavonols, flavan-3-ols,
95 proanthocyanidins, flavones, flavanones, anthocyanins, and isoflavones. We also explored
96 associations of flavonoid-rich wholefoods and beverages including tea, apples/pears, oranges/ other
97 citrus fruits and chocolate.

98

99 **Methods**

100 *Study population*

101 The Blue Mountains Eye Study (BMES) is a population-based cohort study of common eye
102 diseases and other health outcomes in a suburban Australian population located west of Sydney.
103 Study methods and procedures have been described elsewhere [18]. Participants were non-
104 institutionalized residents aged 49 years or older invited to attend a detailed baseline eye
105 examination after a door-to-door census of the study area. Selection bias at baseline was minimized
106 after multiple call-back visits that included door-knocking, telephone reminders and letters at
107 recruitment. During 1992-4, 3654 participants (participation rate of 82.4%) aged >49 years were
108 examined. Of the 3654 examined at BMES, 3501 had data on retinal vessel calibre. Of these, 2821
109 had complete dietary data and hence, were included for final cross-sectional analyses. The study
110 was approved by the Human Research Ethics Committee of the University of Sydney and was
111 conducted adhering to the tenets of the Helsinki Declaration. Signed informed consent was obtained
112 from all participants.

113

114 *Assessment of flavonoid intake*

115 Dietary data were collected using a 145-item self-administered food frequency questionnaire (FFQ).
116 The FFQ was modified for Australian diet and vernacular from an early Willett FFQ [19] and
117 includes reference portion sizes. Participants used a 9-category frequency scale to indicate the usual
118 frequency of consuming individual food items during the past year. Foods listed in the FFQ were
119 categorized into major food categories and subcategories similar to those used for the 1995
120 Australian National Nutrition Survey [20]. Estimates of the flavonoid content of foods in the FFQ
121 were derived from the US Department of Agriculture (USDA) Database for the Flavonoid Content
122 of Selected Foods [21], USDA Database for the Isoflavone Content of Selected Foods [22, 23] and
123 USDA Database for the Proanthocyanidin Content of Selected Foods Flavonoid-rich whole foods
124 were decided upon by choosing the greatest dietary contributors to flavonoid intake for each class
125 [24].

126 The method of computing the flavonoid content of foods was similar to that reported by *Ivey*
127 *et al.* [8]. Specifically, for each food, we computed the intake of each individual flavonoid
128 compound present in the food, the sum of assessed flavonoids for each flavonoid subclass, by
129 summing the individual compounds of each flavonoid subclass, and the sum of all flavonoid
130 intakes, by summing the flavonoid subclasses. A worked example of the flavonoid calculation
131 (using tea as the example) is provided in the online supplementary material. The flavan-3-ol content
132 of foods was considered to represent the average of total flavan-3-ol and proanthocyanidin
133 monomer contents. For foods where only the flavan-3-ol or proanthocyanidin monomer content was
134 available, the single value provided was used to represent the flavan-3-ol content. The
135 proanthocyanidin content of foods was calculated by summing the proanthocyanidin dimers,
136 trimers, 4–6mers, 7–10mers and polymers. Where multiple varieties of a food listed in the FFQ
137 were reported in the databases, the average flavonoid content of all similar varieties was computed,
138 consistent with the descriptors used in the FFQ output. Foods in the FFQ that were not in the
139 flavonoid databases were assumed to contain no flavonoids. Intakes of flavonoid subclasses (in
140 mg/d) were calculated by multiplying the estimated intake (g edible portion/d) from the FFQ, with

141 the flavonoid subclass content (mg/d edible portion) of each food item on the questionnaire. Some
142 of the food items on the FFQ with multiple ingredients (e.g., pizza) were assigned a weighted value
143 on the basis of a USDA standard recipe [17].

144

145 *Assessment of retinal vascular calibre*

146 Detailed methods for grading the calibre of retinal arterioles and venules are described elsewhere
147 [25]. Briefly, at the baseline examination, 30° photographs of the macula, optic disc, and other
148 retinal fields of both eyes were taken, after pupil dilation, using a Zeiss FF3 fundus camera (Zeiss,
149 Oberkochen, Germany). Methods developed by the University of Wisconsin–Madison [26] were
150 used to measure the internal calibre of retinal arterioles and venules from digitized photographs.
151 These were then summarized using established formulas [27] to account for branching patterns and
152 combine individual calibre measures into summary indices, and are presented as the central retinal
153 artery equivalent (CRAE) or central retinal vein equivalent (CRVE), representing the mean calibre
154 of these vessels (Online Supplementary Figure). Intra- and inter-grader reliability of this method
155 was high [27], with quadratic weighted κ values of 0.85 (CRAE) and 0.90 (CRVE) found for inter-
156 grader reliability and between 0.80 to 0.93 and 0.80 to 0.92 for intra-grader reliability of the two
157 graders, respectively. Vessel diameters for right eyes were used in the analyses.

158

159 *Assessment of potential confounders*

160 At face-to-face interviews with trained interviewers, a comprehensive medical history that included
161 information about demographic factors, socio-economic characteristics and lifestyle factors like
162 smoking, was obtained from all participants. History of smoking was defined as never, past, or
163 current smoking. Current smokers included those who had stopped smoking within the past year.
164 Information on physician-diagnosed history of stroke was also obtained. Body mass index (BMI)
165 was calculated as weight divided by height squared (kg/m^2). Blood pressure was measured using

166 standard auscultatory methods. Mean arterial blood pressure (mm Hg) was defined as $0.33 \times$ systolic
167 blood pressure + $0.67 \times$ diastolic blood pressure.

168

169 *Statistical analysis*

170 SAS statistical software (v9.4, SAS Institute, Cary NC) was used for analyses including t-tests, χ^2 -
171 tests and linear regression. Analysis of covariance (general linear model, GLM) was used to assess
172 associations between intakes of total flavonoids, flavonoid subclasses and major individual
173 flavonoid compounds, as well as flavonoid-rich foods and beverages with adjusted means of retinal
174 arteriolar and venular calibre. The associations of intake of dietary flavonoids with retinal vessel
175 calibre was examined in linear regression models and GLM, initially adjusted for age and sex, and
176 then further adjusted for BMI, mean arterial blood pressure, smoking, and history of diagnosed
177 stroke. Additionally, to assess the retinal vessel calibre while avoiding collinearity between
178 arteriolar and venular diameters [28], we adjusted arteriolar diameter for venular diameter, and
179 venular diameter for arteriolar diameter, using the residual method suggested by Willett [29].

180

181 **Results**

182 Study characteristics of the 2821 participants included in cross-sectional analyses are shown in
183 Table 1. Participants in the lowest versus highest quartile of total flavonoid intake were more likely
184 to be younger, smokers and have higher BMI and wider retinal venules (Table 1). Table 2 shows
185 that after adjusting for all potential confounders, increasing isoflavone intake was associated with
186 significantly wider retinal arterioles ($\sim 1.8 \mu\text{m}$ difference; $P_{\text{trend}}=0.01$). After multivariable
187 adjustment, increasing dietary intake of proanthocyanidin (from the first to fourth quartile) was
188 associated with smaller retinal venules: $\sim 2.6 \mu\text{m}$ difference ($P_{\text{trend}}=0.01$). Further, the highest versus
189 lowest quartile of anthocyanidin (multivariable-adjusted $p=0.02$) and flavone (multivariable-
190 adjusted $p=0.05$) intake was associated with significantly narrower retinal venules. Finally, when

191 comparing highest versus lowest quartile of isoflavone intake ~2.0 μm wider retinal venules was
192 observed ($P_{\text{trend}}=0.04$; Table 3).

193 Linear regression analyses showed that each mg/day higher dietary intake of
194 proanthocyanidin was associated with 0.01 ± 0.003 μm narrower retinal venules ($p=0.02$). A
195 marginally significant and inverse association (multivariable-adjusted $p=0.05$) was observed
196 between each mg/day higher intake of anthocyanidin and retinal venular calibre. After
197 multivariable-adjustment, each mg/day higher intake of isoflavone was associated with: 1)
198 0.41 ± 0.17 μm wider retinal arteriolar calibre (multivariable-adjusted $p=0.02$); and 2) 0.51 ± 0.19 μm
199 narrower retinal venular calibre (multivariable-adjusted $p=0.01$).

200 Regarding key flavonoid compounds within each subclass, a significant association was
201 observed between the intake of catechins and retinal venular calibre. Specifically, participants in the
202 highest versus lowest quartile of total catechin intake (i.e. catechin plus epicatechin intake) had
203 narrower retinal venules, but this was marginally significant: 223.6 ± 0.62 versus 225.3 ± 0.63 μm ,
204 respectively (multivariable-adjusted $p=0.05$). We also investigated associations of the key dietary
205 contributors to each flavonoid subclass with retinal vascular calibre (Table 4). After adjusting for
206 potential confounders, participants in the highest versus lowest of tertile of apple/ pear consumption
207 had: 1) narrower retinal venules: 226.1 ± 0.52 versus 223.8 ± 0.57 μm ($P_{\text{trend}}=0.01$); and 2) wider
208 retinal arterioles: 186.2 ± 0.51 versus 187.9 ± 0.51 μm ($P_{\text{trend}}=0.02$). Participants who were in the
209 highest versus lowest quartile of chocolate consumption had ~2.1 μm narrower retinal venules
210 ($p=0.03$; Table 4). No significant associations were observed between the other key flavonoid
211 compounds within each subclass as well as the other flavonoid-rich foods (oranges/ other citrus)
212 and beverages (tea, orange juice and red wine) with retinal vascular calibre (Table 4).

213

214 **Discussion**

215 This study provides novel epidemiological evidence of significant associations between specific
216 flavonoid subclasses and retinal vascular calibre, independent of the confounding effects of age,

217 sex, body mass, blood pressure and history of stroke. Specifically, a high dietary intake of flavonoid
218 subclasses including proanthocyanidin, anthocyanidin, and isoflavone in older adults was associated
219 with beneficial structural variations in the retinal vessel calibre, that is, wider retinal arterioles and
220 narrower retinal venules. Another unique observation was that higher consumption (≥ 150.1 g/day)
221 of apples/pears, a source of catechin in the diet, was independently associated with wider retinal
222 arterioles and narrower retinal venules in older adults. While chocolate consumption (source of
223 epicatechin) was significantly and inversely associated with retinal venular caliber.

224 In the BMES, ~74% of total flavonoid intake was attributed to the flavan-3-ol subclass and
225 ~15% came from the proanthocyanidin subclass, where tea and apples were the major dietary
226 contributors, respectively [30]. While no significant associations were observed with intakes of
227 flavan-3-ol and retinal vessel calibre, modest associations were observed with the intake of
228 proanthocyanidins (catechin oligomers). Furthermore, a significant association was also observed
229 with the intakes of isoflavone. Specifically, higher intake of these two flavonoid subclasses were
230 associated with a beneficial retinal microvascular profile, characterized by wider retinal arterioles
231 and narrower retinal venules. The differing structures and bioactivities of the various flavonoid
232 subclasses, and the ability to accurately assess intakes from food frequency questionnaires could
233 explain the varying associations observed between the individual flavonoid subclasses and retinal
234 vessel calibre in the BMES [8,30]. Even a small structural difference in flavonoids can have a large
235 impact on their bioavailability [31,32], which may explain why associations were observed with
236 isoflavone and proanthocyanidin but not with the other flavonoid subclasses. Nevertheless, we
237 caution that the observed findings could be due to chance. For instance, isoflavone intakes in this
238 cohort were very low (mean intake of 1.29 mg/day) and soy intake (a major contributor to
239 isoflavone intake) was likely to be underestimated in the BMES as there were no specific questions
240 about soy products or tofu.

241 The physiological influence of dietary parameters such as flavonoids and flavonoid-rich foods
242 on the retinal microcirculation is likely to be cumulative, long-term and possibly complex [33].

243 Several published studies have shown that following consumption, dietary flavonoids may
244 contribute to a variety of salutary biological activities in humans [8]. For instance, there is
245 increasing evidence showing that dietary flavonoids can preserve and enhance nitric oxide status
246 and improve endothelial function [9-11]. Reduced oxidative stress and improved endothelial
247 function have previously shown to elicit the dilation of retinal arterioles [34], and thus, could be a
248 potential mechanism mediating the positive association between higher intakes of flavonoid
249 subclasses and retinal arteriolar diameter. There is also evidence that dietary flavonoids can
250 minimize oxidative damage and inflammation [35,36]. Prior studies have documented independent
251 associations between systemic inflammatory markers (e.g. C-reactive protein and leukocyte count)
252 and retinal venular calibre [37]. Hence, it is plausible that reduced inflammation is the mechanistic
253 link between the dietary intake of flavonoid subclasses and retinal venular calibre in older adults.

254 There is evidence that the cardioprotective effects of apples in the diet is due, at least in part,
255 to their high flavonoid content [38]. Prior research has shown that higher apple consumption is
256 associated with lower risk of coronary heart disease mortality and all-cause mortality [39].
257 Flavonoid-rich apples are likely to exert these beneficial effects on vascular health by improving
258 endothelial function and lowering blood pressure, as shown in our prior research [38,40]. Given that
259 lower blood pressure and reduced endothelial dysfunction are known to be associated with wider of
260 retinal arterioles and narrower of retinal venules [12] (i.e. favourable structural changes); greater
261 consumption of apples/ pears by BMES participants could have contributed to the observed
262 differences in retinal vascular calibre through pathways that involve blood pressure and/or
263 endothelial function. Chocolate which is another flavonoid-rich food was also significantly
264 associated with retinal venular calibre (i.e. ~2.1 μm narrower diameter) in the BMES. This is in line
265 with the published literature [41,42] that showed chronic intake of chocolate was associated with
266 beneficial effects on cardiovascular health - including BP and flow mediated dilatation.

267 It is now known that retinal arteriolar and venular calibre are associated with clinical
268 outcomes such as stroke and coronary heart disease, independent of blood pressure and other

269 vascular risk factors [12,43]. For example, narrower retinal arteriolar calibre predicts clinical stroke
270 particularly in people with diabetes, and coronary heart disease mortality in women [44]. While
271 wider retinal venular calibre is associated with incident coronary heart disease [12]. Our study
272 findings therefore suggest that the benefits of dietary flavonoids on risk of CVD and on other
273 outcomes related to vascular health, including hypertension and hyperglycemia [45,46], could
274 involve the microvasculature as an intermediate pathway. Further longitudinal cohort studies are
275 warranted to further investigate this hypothesis.

276 It is important to note that the difference in retinal vascular calibre across quartiles of
277 flavonoid subclass intake was relatively modest ($\sim 2.0 \mu\text{m}$). However, even these modest reductions
278 in retinal vessel diameter could still have clinical relevance. Previous studies indicated that even a
279 $1.1 \mu\text{m}$ difference in retinal arteriolar diameter was associated with a 10-mm Hg higher systolic
280 blood pressure [47]. Further, it is plausible that any measurable change to the retinal
281 microvasculature (i.e. subtle retinal arteriolar widening) could result in meaningful differences in
282 CVD risk, given the many studies showing a link between structural changes in retinal arterioles
283 and CVD events and pathology [12]. Therefore, findings from the BMES provide further support
284 for the development of health strategies focusing on increasing the intake of dietary flavonoids and
285 flavonoid-rich foods such as apples and chocolate, which could have salutary effects on both the
286 micro- and macro-vasculature.

287 The key strengths of this study are its relatively large population-base sample; the availability
288 of robust information on potential covariates; the use of a validated FFQ to establish dietary intakes;
289 and a standardized protocol to obtain computer-assisted measurements of retinal vascular calibre
290 from digitized fundus photographs. However, there are limitations that require discussion. First, the
291 cross-sectional design of this study precludes the establishment of causality, although, the most
292 likely direction of association is that dietary intakes of flavonoids influences the retinal
293 microvasculature, because the reverse direction of effect (retinal vasculature changes influencing
294 dietary intake of flavonoids) is unlikely. Second, the FFQ was not designed specifically for

295 polyphenols and the database used for the estimation of flavonoid content of foods is based on US
296 data, thus, the regional variation in flavonoid content of foods was not able to considered in the
297 present study [31]. Finally, we cannot discount the influence of unmeasured or inadequately
298 measured confounding factors on observed associations.

299 In summary, higher dietary intake of specific flavonoid subclasses and greater consumption of
300 flavonoid-rich apple/pear was associated with a beneficial influence on the retinal microvascular
301 health of older adults. These epidemiological data are important as changes to the retinal
302 microvasculature is a marker of microcirculatory health [1,2,4], and the presence of these retinal
303 microvascular signs would support future development of targeted interventions involving greater
304 dietary intakes of flavonoids and flavonoid-rich foods.

305

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308 JRL, GL, NPB, CPB, and GB: conducted the research; GB: analyzed data or performed statistical
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444 **TABLE 1** Study characteristics of Blue Mountains Eye Study participants (n=2821) stratified by
 445 intakes of dietary flavonoids.

Characteristics	Intake of total dietary flavonoid (mg/day)				<i>P</i> -value ^a
	1 st quartile (≤317.9) n=704	2 nd quartile (318.2- 796.0) n=705	3 rd quartile (796.0- 1208.1) n=705	4 th quartile (≥1208.2) n=705	
Age	63.5 (8.9)	65.4 (9.4)	66.4 (9.4)	65.5 (8.8)	<0.0001
Male sex	329 (46.7)	308 (43.7)	320 (45.4)	285 (40.4)	0.09
Current smoking	127 (18.8)	95 (13.8)	84 (12.2)	78 (11.3)	0.0003
Body mass index, <i>kg/m</i> ²	26.5 (4.7)	26.4 (4.6)	25.9 (4.2)	25.9 (4.3)	0.01
Mean arterial BP, <i>mm</i> <i>Hg</i>	103.3 (12.4)	104.7 (12.3)	104.8 (12.8)	104.2 (11.4)	0.09
History of diagnosed stroke	41 (5.8)	34 (4.8)	31 (4.4)	20 (2.8)	0.05
Retinal vascular calibre, <i>μm</i>					
Arteriolar calibre	188.4 (18.9)	187.2 (17.8)	186.8 (17.7)	186.4 (18.9)	0.19
Venular calibre	227.0 (20.9)	224.2 (20.0)	224.9 (21.3)	223.8 (21.2)	0.02

446 Data are presented as means ± SD or n (%).

447 ^aUnadjusted *p*-values from test for heterogeneity across quartiles of intake.

448 **TABLE 2** Cross-sectional association between tertiles of dietary flavonoid and retinal arteriolar
 449 calibre among study participants (n=2821)

Dietary intake mg/day (range)	Mean retinal arteriolar calibre (SE), μm	
	Age-sex adjusted	Multivariable-adjusted ^a
Total flavonoids		
1 st quartile (≤ 317.9), n=705	187.2 (0.55)	187.2 (0.56)
2 nd quartile (318.2-796.0), n=706	187.5 (0.55)	187.6 (0.55)
3 rd quartile (796.0-1208.1), n=706	186.9 (0.55)	186.9 (0.55)
4 th quartile (≥ 1208.2), n=705	186.8 (0.55)	186.8 (0.55)
<i>P</i> for trend	0.62	0.59
Flavonols		
1 st quartile (≤ 18.0), n=705	187.4 (0.55)	187.2 (0.55)
2 nd quartile (18.0-34.2), n=706	187.7 (0.55)	187.7 (0.55)
3 rd quartile (34.2-46.6), n=706	186.9 (0.55)	187.0 (0.55)
4 th quartile (≥ 46.6), n=705	186.7 (0.55)	186.7 (0.55)
<i>P</i> for trend	0.39	0.44
Flavone		
1 st quartile (≤ 0.57), n=705	187.2 (0.55)	186.7 (0.55)
2 nd quartile (0.58-1.03), n=706	187.1 (0.55)	187.1 (0.55)
3 rd quartile (1.03-1.52), n=706	187.3 (0.55)	187.5 (0.55)
4 th quartile (≥ 1.53), n=705	186.9 (0.55)	187.2 (0.55)
<i>P</i> for trend	0.78	0.49
Flavan-3-ol		
1 st quartile (≤ 243.1), n=706	187.4 (0.55)	187.3 (0.56)
2 nd quartile (243.4-729.0), n=706	187.4 (0.55)	187.6 (0.55)
3 rd quartile (729.1-1149.6), n=706	186.8 (0.55)	186.9 (0.55)

4 th quartile (≥ 1149.6), n=706	186.9 (0.55)	186.8 (0.55)
<i>P</i> for trend	0.41	0.34
Anthocyanidin		
1 st quartile (≤ 2.97), n=705	187.5 (0.55)	187.3 (0.56)
2 nd quartile (2.98-5.58), n=706	188.3 (0.55)	188.5 (0.55)
3 rd quartile (5.58-12.32), n=706	186.1 (0.55)	186.2 (0.56)
4 th quartile (≥ 12.34), n=706	186.6 (0.55)	186.5 (0.55)
<i>P</i> for trend	0.15	0.13
Flavanone		
1 st quartile (≤ 6.59), n=706	187.4 (0.55)	186.9 (0.55)
2 nd quartile (6.64-24.58), n=706	187.2 (0.55)	187.1 (0.55)
3 rd quartile (24.71-47.39), n=706	186.8 (0.55)	187.1 (0.55)
4 th quartile (≥ 47.42), n=706	187.0 (0.55)	187.4 (0.55)
<i>P</i> for trend	0.57	0.62
Isoflavone		
1 st quartile (≤ 0.71), n=706	186.2 (0.55)	186.3 (0.56)
2 nd quartile (0.71-1.00), n=706	186.3 (0.55)	186.3 (0.55)
3 rd quartile (1.00-1.42), n=706	187.7 (0.55)	187.7 (0.55)
4 th quartile (≥ 1.42), n=706	188.2 (0.55)	188.1 (0.55)
<i>P</i> for trend	0.003	0.01
Proanthocyanidin		
1 st quartile (≤ 69.5), n=706	187.1 (0.55)	186.8 (0.56)
2 nd quartile (69.6-117.6), n=706	186.9 (0.55)	187.1 (0.55)
3 rd quartile (117.6-175.4), n=706	186.7 (0.55)	186.6 (0.55)
4 th quartile (≥ 175.4), n=706	187.7 (0.55)	187.9 (0.55)
<i>P</i> for trend	0.41	0.21

450 ^aFurther adjusted for fellow retinal vascular calibre, body mass index, smoking, mean arterial blood
451 pressure, and history of diagnosed stroke.

452 **TABLE 3** Cross-sectional association between tertiles of dietary flavonoid intake and retinal
 453 venular calibre among study participants (n=2821)

Dietary intake (mg/day)	Mean retinal venular calibre (SE), μm	
	Age-sex adjusted	Multivariable-adjusted ^a
Total flavonoids		
1 st quartile (≤ 317.9)	225.6 (0.63)	225.3 (0.64)
2 nd quartile (318.2-796.0)	224.1 (0.63)	223.9 (0.62)
3 rd quartile (796.0-1208.1)	225.1 (0.63)	225.3 (0.63)
4 th quartile (≥ 1208.2)	224.3 (0.63)	224.6 (0.62)
<i>P</i> for trend	0.16	0.47
Flavonols		
1 st quartile (≤ 18.0)	225.1 (0.63)	225.1 (0.63)
2 nd quartile (18.0-34.2)	224.8 (0.63)	224.8 (0.63)
3 rd quartile (34.2-46.6)	224.8 (0.63)	224.9 (0.63)
4 th quartile (≥ 46.6)	224.3 (0.63)	224.6 (0.63)
<i>P</i> for trend	0.38	0.74
Flavone		
1 st quartile (≤ 0.57)	226.0 (0.63)	225.9 (0.63)
2 nd quartile (0.58-1.03)	224.7 (0.63)	224.7 (0.62)
3 rd quartile (1.03-1.52)	224.1 (0.62)	224.2 (0.63)
4 th quartile (≥ 1.53)	224.3 (0.63)	224.2 (0.63) ^b
<i>P</i> for trend	0.07	0.07
Flavan-3-ol		
1 st quartile (≤ 243.1)	225.4 (0.63)	225.1 (0.64)
2 nd quartile (243.4-729.0)	224.8 (0.63)	224.4 (0.62)
3 rd quartile (729.1-1149.6)	224.3 (0.63)	224.4 (0.63)

4 th quartile (≥ 1149.6)	224.6 (0.63)	225.0 (0.62)
<i>P</i> for trend	0.33	0.93
Anthocyanidin		
1 st quartile (≤ 2.97)	226.4 (0.63)	225.1 (0.63)
2 nd quartile (2.98-5.58)	224.5 (0.63)	224.8 (0.63)
3 rd quartile (5.58-12.32)	224.3 (0.62)	225.3 (0.63)
4 th quartile (≥ 12.34)	223.9 (0.63)	223.8 (0.62) ^c
<i>P</i> for trend	0.05	0.09
Flavanone		
1 st quartile (≤ 6.59)	225.5 (0.63)	225.5 (0.63)
2 nd quartile (6.64-24.58)	223.7 (0.63)	223.7 (0.62)
3 rd quartile (24.71-47.39)	224.8 (0.63)	224.8 (0.63)
4 th quartile (≥ 47.42)	225.0 (0.63)	225.1 (0.63)
<i>P</i> for trend	0.87	0.74
Isoflavone		
1 st quartile (≤ 0.71)	225.5 (0.63)	225.6 (0.63)
2 nd quartile (0.71-1.00)	224.8 (0.63)	224.7 (0.63)
3 rd quartile (1.00-1.42)	225.2 (0.63)	225.1 (0.62)
4 th quartile (≥ 1.42)	223.7 (0.63)	223.6 (0.62)
<i>P</i> for trend	0.06	0.04
Proanthocyanidin		
1 st quartile (≤ 69.5)	226.9 (0.62)	226.5 (0.63)
2 nd quartile (69.6-117.6)	224.8 (0.62)	224.6 (0.62)
3 rd quartile (117.6-175.4)	223.8 (0.62)	224.0 (0.63)
4 th quartile (≥ 175.4)	223.5 (0.62)	223.9 (0.62)
<i>P</i> for trend	0.0001	0.01

454 ^a Further adjusted for fellow retinal vascular calibre, body mass index, smoking, mean arterial blood
455 pressure, and history of diagnosed stroke.
456 ^b Significantly different when compared to the 1st tertile of intake: p=0.05
457 ^c Significantly different when compared to the 1st tertile of intake: p=0.02

458 **TABLE 4** Cross-sectional linear association between intakes of flavonoid-rich foods and
 459 retinal vascular calibre among study participants (n=2821)

Dietary intake (g/day)	Adjusted mean retinal vascular calibre (SE), μm	
	Retinal arteriolar calibre ^a	Retinal venular calibre ^a
Apple/ Pear		
1 st tertile (≤ 10.4)	186.2 (0.46)	226.1 (0.52)
2 nd tertile (21.1-64.7)	187.4 (0.47)	224.1 (0.54)
3 rd tertile (≥ 150.5)	187.9 (0.51)	223.8 (0.57)
<i>P</i> for trend	0.02	0.01
Oranges		
1 st tertile (≤ 2.50)	186.7 (0.51)	225.1 (0.58)
2 nd tertile (8.75-53.75)	187.0 (0.43)	224.7 (0.48)
3 rd tertile (≥ 98.75)	187.8 (0.52)	224.5 (0.59)
<i>P</i> for trend	0.12	0.59
Orange juice		
1 st tertile (≤ 2.50)	186.8 (0.42)	224.8 (0.48)
2 nd tertile (8.75-17.50)	187.2 (0.61)	224.5 (0.69)
3 rd tertile (≥ 53.75)	187.4 (0.46)	224.8 (0.52)
<i>P</i> for trend	0.37	0.95
Tea		
1 st tertile (≤ 250.0)	187.3 (0.46)	224.8 (0.52)
2 nd tertile (625.0-625.0)	187.2 (0.48)	224.3 (0.54)
3 rd tertile (≥ 1000.0)	186.8 (0.51)	225.2 (0.58)
<i>P</i> for trend	0.46	0.69
Red wine		

1 st tertile (≤ 0.00)	187.5 (0.36)	225.0 (0.40)
2 nd tertile (2.40-2.40)	187.5 (0.48)	225.1 (0.50)
3 rd tertile (≥ 8.40)	186.5 (0.45)	224.3 (0.51)
<i>P</i> for trend	0.07	0.26
Chocolate		
1 st quartile (≤ 0.00)	187.5 (0.63)	225.9 (0.72)
2 nd quartile (1.00-1.00)	186.9 (0.48)	224.9 (0.55)
3 rd quartile (3.50-3.50)	187.0 (0.62)	224.6 (0.70)
4 th quartile (≥ 7.00)	187.3 (0.52)	223.9 (0.59) ^b
<i>P</i> for trend	0.78	0.06

460 ^a Adjusted for age, sex, fellow retinal vascular calibre, body mass index, smoking, mean
461 arterial blood pressure, and history of diagnosed stroke.

462 ^b Significantly different when compared to the 1st tertile of intake: $p=0.03$