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Bamini Gopinath

Gerald Liew

Joshua Lewis
Edith Cowan University

Nicola P. Bondonno

Catherine P. Bondonno
Edith Cowan University

See next page for additional authors

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Authors

Bamini Gopinath, Gerald Liew, Joshua Lewis, Nicola P. Bondonno, Catherine P. Bondonno, George Burlutsky, Jonathan M. Hodgson, and Paul Mitchell

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Associations between dietary flavonoids and retinal microvasculature in older adults

Running title: Flavonoids and retinal vessels

Bamini Gopinath[1]

Gerald Liew[1]

Joshua R Lewis[2,3,4]

Nicola P Bondonno[5]

Catherine P Bondonno[3,4]

George Burlutsky[1]

Jonathan M Hodgson[3,4]

Paul Mitchell[1]

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

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

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

[4] Medical School, University of Western Australia, Perth, WA, Australia.

[5] School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia.

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:

BMES - Blue Mountains Eye Study

CRAE - Central Retinal Artery Equivalent

CRVE - Central Retinal Vein Equivalent

CVD - Cardiovascular Disease

FFQ - Food-Frequency Questionnaire

GLM - General Linear Model

USDA - US Department of Agriculture

Abstract

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

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

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

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

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

Introduction

There is increasing evidence that measures of retinal microvascular health are associated with early stages of subclinical cardiovascular disease (CVD) and are predictive of clinical CVD events [1-3]. The retinal microvasculature may provide an integrated assessment of the microvascular consequences of exposure to vascular insults and may be a valuable prognostic tool in predicting future CVD risk [1,2,4]. A beneficial retinal microvascular profile is typically characterized by wider retinal arterioles and narrower retinal venules [1,2,4]. We previously showed that individual dietary factors associated with CVD risk factors and related events, such as dairy foods, carbohydrates and overall diet quality, were also independently related to characteristics of the retinal microvasculature [5-7].

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

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

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]. Participants were non-institutionalized residents aged 49 years or older invited to attend a detailed baseline eye examination after a door-to-door census of the study area. Selection bias at baseline was minimized after multiple call-back visits that included door-knocking, telephone reminders and letters at recruitment. During 1992-4, 3654 participants (participation rate of 82.4%) aged >49 years were examined. Of the 3654 examined at BMES, 3501 had data on retinal vessel calibre. Of these, 2821 had complete dietary data and hence, were included for final cross-sectional analyses. The study was approved by the Human Research Ethics Committee of the University of Sydney and was conducted adhering to the tenets of the Helsinki Declaration. Signed informed consent was obtained from all participants.

Assessment of flavonoid intake

Dietary data were collected using a 145-item self-administered food frequency questionnaire (FFQ). The FFQ was modified for Australian diet and vernacular from an early Willett FFQ [19] 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 [20]. 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 [21], USDA Database for the Isoflavone Content of Selected Foods [22, 23] and USDA Database for the Proanthocyanidin Content of Selected Foods Flavonoid-rich whole foods were decided upon by choosing the greatest dietary contributors to flavonoid intake for each class [24].

The method of computing the flavonoid content of foods was similar to that reported by *Ivey et al.* [8]. 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 subclass, by summing the individual compounds of each flavonoid subclass, and the sum of all flavonoid intakes, by summing the flavonoid subclasses. A worked example of the flavonoid calculation (using tea as the example) is provided in the online supplementary material. 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 subclasses (in mg/d) were calculated by multiplying the estimated intake (g edible portion/d) from the FFQ, with

the flavonoid subclass 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 [17].

Assessment of retinal vascular calibre

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

Assessment of potential confounders

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

standard auscultatory methods. Mean arterial blood pressure (mm Hg) was defined as 0.33×systolic blood pressure + 0.67×diastolic blood pressure.

Statistical analysis

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

Results

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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TABLE 1 Study characteristics of Blue Mountains Eye Study participants (n=2821) stratified by intakes of dietary flavonoids.

Characteristics	Intake of total dietary flavonoid (mg/day)				
	1st quartile (≤317.9) n=704	2nd quartile (318.2-796.0) n=705	3rd quartile (796.0-1208.1) n=705	4th quartile (≥1208.2) n=705	*P*-value [a]
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, kg/m^2	26.5 (4.7)	26.4 (4.6)	25.9 (4.2)	25.9 (4.3)	0.01
Mean arterial BP, *mm Hg*	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, *μm*					
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

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

[a] Unadjusted 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)

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

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

[a] Further adjusted for fellow retinal vascular calibre, body mass index, smoking, mean arterial blood pressure, and history of diagnosed stroke.

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

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

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

[a] Further adjusted for fellow retinal vascular calibre, body mass index, smoking, mean arterial blood pressure, and history of diagnosed stroke.
[b] Significantly different when compared to the 1st tertile of intake: p=0.05
[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)

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

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