Is the Association Between Smoking and the Retinal Venular Diameter Reversible Following Smoking Cessation?

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Purpose. Wider retinal venular caliber is shown to be associated with an increased risk of stroke, and smoking is associated with a wider retinal venular caliber. However, the impact of smoking cessation on the retinal vessels has not been previously reported. We examined this issue in an adult cohort of atomic bomb survivors.

Methods. In the Adult Health Study of Japanese atomic bomb survivors, 1664 subjects had retinal photographs taken from 2006 to 2008. The central retinal artery and vein equivalents (CRAE and CRVE) were calculated using a semiautomated software program. Multiple surveys have assessed the effects of smoking since 1963. The associations between smoking, the time since cessation, and the retinal vessel caliber were determined using linear mixed effects models.

Results. The CRVE was associated with an increased number of cigarettes smoked per day among women after adjusting for potential confounding factors (age, sex, blood pressure, hypertensive medications, white blood cell count, diabetes, body mass index, lipids, and radiation dose). Females who smoked 10 cigarettes per day had a 6.9-μm wider mean CRVE ($P = 0.001$) than nonsmokers. Females who had stopped smoking for 10 or more years had a mean CRVE similar to those who had never smoked (191.8 vs. 194.4 μm; $P = 0.23$). These associations were not observed in males or for CRAE.

Conclusions. Wider retinal venular caliber is associated with smoking in Japanese females; however, this association becomes nonsignificant after 10 or more years of smoking cessation, suggesting that the impact of smoking on retinal venular dilation is reversible following long-term smoking cessation.

Keywords: retinal vessels, smoking, microcirculation, arterioles, venules

Cigarette smoking is one of the most important modifiable risk factors for preventing systemic cardiovascular diseases, such as stroke,[1] via mechanisms that include alterations in vascular compliance, elasticity, endothelial function, and increased oxidative stress.[2,3] Furthermore, cardiovascular diseases account for more than one-third of deaths in cigarette smokers.[4]

The retinal vasculature is unique, as it can be observed directly and noninvasively in vivo. Retinal images have been quantitatively assessed with semiautomated computer software programs and used to demonstrate an association between the vascular caliber and cardiovascular disease.[5] For instance, the Beaver Dam Study and Blue Mountain Study showed that narrower retinal arteriolar and wider retinal venular caliber predicted the risk of cardiovascular death.[6,7]

Cigarette smoking has been linked to a wider retinal venular caliber in a number of population-based studies.[8,9] The gradient association pattern between the dose of smoking and the retinal vasculature is unclear, based on findings from the few studies that have examined this relationship and included evaluating detailed smoking patterns and dosages.[9] Most previous studies are cross-sectional and have limited numbers of subjects to make comparisons among (or in strata of) current, past, and nonsmokers.[8,10,11] Moreover, there are limited data documenting whether retinal venular dilation is reversible after smoking cessation, and, if so, how long it takes for this parameter to normalize to the levels observed in never smokers.

The purpose of the current report was to examine whether the relationship between smoking and the retinal vascular

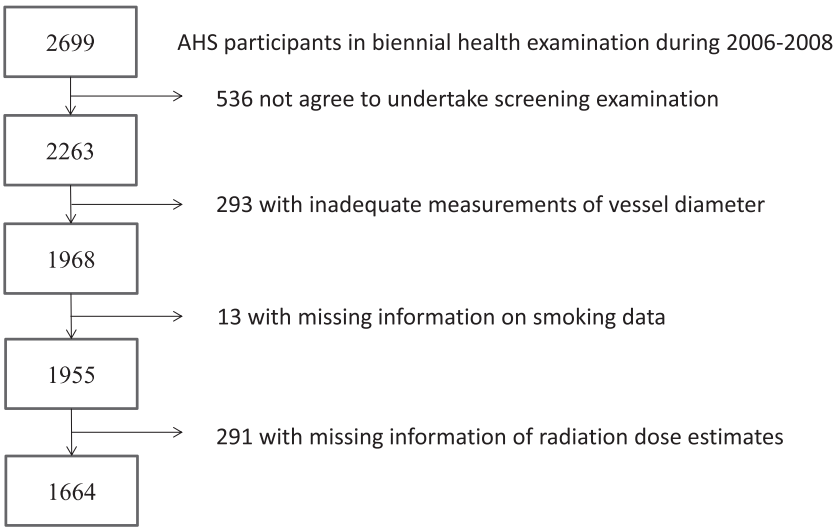

Figure. Number of subjects.

caliber is reversible after smoking cessation in participants of the Adult Health Study (AHS).

Methods

Subjects

The AHS is a cohort study that includes a biannual health examination program conducted since 1958 among survivors of the atomic bombings of Hiroshima and Nagasaki, Japan. In 1950, the Radiation Effects Research Foundation (RERF) (formerly the Atomic Bomb Casualty Commission) established a Life Span Study (LSS) of 120,321 survivors of the atomic bombings of Hiroshima and Nagasaki. In 1958, a series of comprehensive physical examinations was launched in association with enrollment in the AHS, consisting of 19,961 LSS participants.[12] The participation rate has ranged from 70% to 90% throughout the examination cycle. A total of 2699 AHS participants were nominated at the current examination cycle. Fundoscopic examinations were performed on 2263 eligible participants, after obtaining written informed consent and ethical approval from the RERF Ethics Committee for this study conducted from 2006 to 2008, in line with the provisions of the Declaration of Helsinki. A total of 1955 eligible subjects were involved in this study, after excluding 293 subjects with inadequate measurements of vessel diameters and 13 subjects with missing information on detailed smoking habits. A total of 291 persons were excluded due to a lack of eye radiation dose estimates. A total of 1664 subjects were evaluated in this study (Figure).

Measurement of the Retinal Vascular Caliber

Digital fundus color photographs were taken through undilated pupils using a fundus camera (TRC-NW200; Topcon, Tokyo, Japan). A semiautomated computer imaging program (Retinal Analysis-IVAN, University of Wisconsin, Madison, WI) was used to measure and summarize the retinal vascular calibers by a trained grader who was masked to the participant characteristics. The arteriolar and venular diameters were measured in a specific zone (0.5 to 1.0 disc diameters away from the optic disc margin). The largest six-vessel measurements were used to calculate the central retinal artery and vein equivalents (CRAE and CRVE) for both eyes. Retinal images with fewer than six identifiable largest arterioles or venules were excluded from the analysis.[5,13] The intragrader reproducibility of the measurements was confirmed by repeating the masked measurements twice on a subsample of 100 images; the intraclass correlation coefficient was high (>0.90).

Cigarette Smoking

We collected information regarding lifelong smoking behaviors periodically since 1963 using multiple interviews and mail surveys,[14] including detailed information on long-term cigarette smoking habits (e.g., the number of cigarettes smoked per day and duration of smoking or smoking cessation). The interviews were conducted using open questions. Because the participants were asked about the number of cigarettes smoked as a daily custom, many of the answers were provided in multiples of 10. Based on the self-reported data collected during interviews at examinations (1986–2008), past interviews (1963, 1965, 1968), and mail surveys (1965, 1969, 1978), we defined current smokers as those who were smoking at the time of examination (2006–2008) and further categorized those who currently smoked as smoking "<10," "≥ 10 to <20" or "≥ 20 cigarettes/day." In brief, the subjects with no indications of a smoking habit in any interviewing data were considered to be "never smokers." "Past smokers" were defined as subjects with any indications of a smoking habit between 1963 and 2005 who were not smoking in 2006–2008. Furthermore, for the purpose of investigating the effects of smoking cessation, we classified 1497 persons who were recorded to be "nonsmokers" at the last examination during the cycle of 2006–2008 but were recoded as smokers between 1963 and 2005 into two groups: nonsmokers for ≤ 10 years or >10 years.

Systemic Assessment of Concomitant Characteristics

The concomitant data included age, sex, mean arterial blood pressure (MAP), white blood cell count (WBC), diabetes, body mass index (BMI), total cholesterol (T-CHO), triglycerides, radiation dose, and city at the time of the bombings. Blood pressure was measured in a sitting position at the right brachial artery with a sphygmomanometer. The MAP was calculated as the sum of diastolic pressure plus one-third of the pulse

Table 1. Characteristics of the AHS Participants (2006–2008) According to Current Smoking Status

All Subjects	Current Cigarettes Smoked per Day				*P** for Trend
	Nonsmoker	>0 to <10	≥10 to <20	≥20	
No. of subjects	1497	26	62	79	
Mean CRAE, μm	129.9	129.6	131.9	134.4	
Mean CRVE, μm	195.8	203.1	205.6	204.8	
Age, y	75.1 ± 6.5	73.7 ± 6.5	72.8 ± 5.8	69.4 ± 5.2	<0.0001
MAP, mm Hg	93.0 ± 11.0	91.8 ± 9.3	91.5 ± 11.5	93.5 ± 11.9	0.16
Radiation dose, Gy	0.48 ± 0.78	0.3 ± 0.67	0.5 ± 0.74	0.5 ± 0.66	0.85
Triglycerides, mg/dL	116.8 ± 70.7	116.6 ± 59.0	131.5 ± 91.5	139.6 ± 88.3	0.015
T-CHO, mg/dL	206.2 ± 33.4	196.1 ± 25.3	194.9 ± 29.0	203.1 ± 34.3	0.009
WBC, 10^2/mm^3	54.8 ± 14.9	57.6 ± 19.1	66.6 ± 19.6	67.5 ± 15.3	<0.0001
BMI, kg/m^2	22.9 ± 3.4	23.0 ± 2.7	22.1 ± 3.5	22.4 ± 2.9	0.02
Hypertension medication, %	46.4	38.5	50.0	34.2	0.14
Diabetes, %	0.6	1.1	0.5	1.2	0.0004

Continuous variables are shown as the average ± SD.
* Adjusted for age.

pressure (mm Hg), and BMI was defined as the weight divided by the height squared (kg/m^2). The diagnosis of diabetes was made according to the American Diabetes Association criteria of a nonfasting plasma glucose level of 200 mg/dL or higher, an HbA1c level of 6.5% or higher, the use of medications for the treatment of diabetes, or a history of diabetic retinopathy.[15] The levels of nonfasting triglycerides in the serum were measured enzymatically. The measurement of nonfasting T-CHO is described elsewhere.[16] The weighted absorbed eye dose of radiation, in units of gray (Gy), was estimated based on a 2002 revised dosimetry system that takes into account the effects of building and environmental shielding and physical location.[17]

Statistical Methods

The retinal vascular calibers (CRAE and CRVE) were examined as continuous dependent variables. We used an analysis of covariance or logistic regression analysis to describe the sex-specific relationships between smoking and the concomitant study factors with adjustment for age. The associations between smoking and the CRAE and CRVE in both eyes were analyzed using a linear mixed effects model with a random intercept for each subject, adjusted for age, sex, city, MAP, hypertensive medications (yes or no), WBC, diabetes history (yes or no), BMI, cholesterol, triglycerides, and radiation dose.[18] The linear mixed effects model provided a means to simultaneously model the CRAE and CRVE measurements of the left and right eyes, taking the correlation between the two eyes into account. Each subject contributed data for one or both eyes. In the multivariable-adjusted models, subjects with missing measurements in covariables were excluded from the analysis. Because a strong association between age and the retinal calibers was expected, nonlinearity of the effects of age was accounted for by adding a squared term for age in the model if necessary. Tests for trends were also conducted with respect to the current number of cigarettes smoked per day as a continuous variable. In modeling trends across strata of smoking history that reflect increasing exposure to cigarette smoking (i.e., never smoked, stopped smoking ≥10 years, stopped smoking <10 years, and current smoking), the smoking status was modeled as an ordinal variable. Because stronger smoking effects were observed in females in a previous RERF study,[19] it is possible that different effects on the retinal vascular caliber are observed between males and females. We investigated the interaction between smoking and sex in association with the retinal vessel caliber and considered the interaction to be significant for P less than 0.05. When the interaction was statistically significant, analyses stratified according to sex were conducted. All reported P values were based on two-sided tests of significance. The statistical modeling and testing used the SAS software program (version 9.3; SAS Institute, Inc., Cary, NC). With regard to missing retinal vessel calibers in either the left or right eyes, the missing mechanism was treated as missing-at-random (MAR) in the MIXED procedure.

Table 2. Associations of Retinal Vessel Diameters

All Subjects	Mean Difference in CRAE, μm	*P*	Mean Difference in CRVE, μm	*P*
Smoking per 1 number/day	0.07 [−0.04–0.19]	0.2	**0.18 [0.03–0.34]***	**0.02**
Women vs. men	**1.98 [0.55–3.40]***	**0.01**	**−2.03 [−3.99 to −0.08]***	**0.04**
Age per 1 years old	**−0.25 [−0.35 to −0.14]***	**<0.0001**	**−0.41 [−0.55–0.27]***	**<0.0001**
Age^2 per 1 (year old)^2			**−0.03 [−0.04 to −0.01]***	**0.001**
MAP per mm Hg	**−0.21 [−0.27 to −0.15]***	**<0.0001**	**−0.15 [−0.24 to −0.07]***	**0.0002**
WBC per 10^2/mm^3	0.02 [−0.02–0.06]	0.35	**0.11 [0.05–0.17]***	**0.0003**
Diabetes present vs. absent	−0.45 [−1.98–1.08]	0.56	1.02 [−1.06–3.12]	0.34
BMI per kg/m^2	−0.05 [−0.25–0.16]	0.65	0.25 [−0.03–0.53]	0.07
T-CHO per mg/dL	0.01 [−0.01–0.03]	0.35	0.01 [−0.02–0.04]	0.59
Triglycerides per mg/dL	−0.004 [−0.01–0.01]	0.43	0.002 [−0.01–0.01]	0.8
Hypertension medication present vs. absent	−0.3 [−1.68–1.07]	0.67	**1.93 [0.06–3.79]***	**0.04**

The 95% confidence interval is in brackets. Values in bold indicate significance.
* Significant ($P < 0.05$). Adjusted for city and weighted absorbed eye dose of radiation to the retinal vessel calibers.
Age^2, a centered squared term for age.

Table 3. Mean Retinal Vessel Caliber Differences According to the Smoking Strata

	CRAE, μm	Difference Relative to Never Smoker*	*P*	CRVE, μm	Difference Relative to Never Smoker*	*P*
All subjects						
Never smoker	128.33	—	—	195.8	—	—
>0 to <10 smoked/day	128.45	−0.11 [−5.31–5.54]	0.97	197.7	1.94 [−5.28–9.16]	0.60
≥10 to <20 smoked/day	129.34	−1.01 [−2.64–4.66]	0.59	200.8	5.00 [−0.04–10.0]	0.05
≥20 smoked/day	131.73	3.40 [−0.03–6.82]	0.05	**201.7**	**5.96 [1.21–10.7]**	**0.01**
Test for trend	0.06			**0.002**		
Men						
Never smoker	126.6	—	—	197.8	—	—
>0 to <10 smoked/day	124.8	0.11 [−5.31–5.54]	0.97	197.2	−0. 61 [−10.0–8.78]	0.90
≥10 to <20 smoked/day	127.6	1.01 [−2.64–4.66]	0.59	201.3	3.54 [−2.83–9.90]	0.28
≥20 smoked/day	131.3	3.40 [−0.03–6.82]	0.05	201.4	3.59 [−2.13–9.32]	0.22
Test for trend	0.08			0.09		
Women						
Never smoker	129.1	—	—	194.5	—	—
>0 to <10 smoked/day	133.3	4.22 [−4.07–12.5]	0.32	201.4	6.97 [−4.46–18.4]	0.23
≥10 to <20 smoked/day	133.1	4.06 [−2.36–10.5]	0.22	204.0	**9.52 [0.37–18.7]***	**0.04**
≥20 smoked/day	132.7	3.62 [−4.49–11.7]	0.38	211.4	**16.9 [5.56–28.3]***	**0.004**
Test for trend	0.29			**0.002**		

P value versus never smoker. Bold values indicate significance. —, no values available.

* Adjusted for age, sex, city, MAP, WBC, diabetes, BMI, cholesterol, triglycerides, hypertensive medications, and weighted absorbed eye dose of radiation.

Results

Characteristics of the Study Participants

Of the 1664 subjects, 167 were current smokers (males: 129, females: 38), 492 were past smokers (males: 385, females: 107), and 956 had never smoked (males: 84, females: 872). Of the past smokers, 131 males (26.6%) and 25 females (5.1%) had stopped smoking within 10 years and 254 males (51.6%) and 82 females (16.7%) had stopped smoking for more than 10 years. A total of 49 subjects were excluded from the past smoking status analysis in Table 4 because of a lack of past smoking data.

Associations of Cigarette Smoking and Other Potential Risk Characteristics

Table 1 describes the associations between cigarette smoking and the other possible confounders measured in the study. Among all subjects, the mean age of the study sample was 74.7 ± 6.5 (SD) years, with 64% females. The number of cigarettes smoked per day declined as the cohort members became older, and the mean WBC level significantly increased in association with increasing numbers of cigarettes smoked per day in both males and females. An increased number of cigarettes smoked per day was also associated with a higher level of triglycerides and a lower BMI. The T-CHO levels and incidence of diabetes were significantly different across the smoking strata, although the tendency was not clear. In both males and females, a younger age and higher WBC were related to an increased number of cigarettes smoked per day.

Associations Between Smoking and the Retinal Vessel Diameters

Table 2 shows the associations between the current smoking status (current smokers versus current nonsmokers) and risk factors and the CRAE and CRVE based on the linear mixed-effects models. In the multivariate analysis of all subjects, both the CRAE and CRVE increased in association with an increasing number of cigarettes smoked per day. There were no significant associations between smoking and CRAE ($P = 0.20$). In addition, men, age, and MAP were associated with narrower CRAE, whereas smoking, men, lower MAP, WBC, HT medications, younger age, and the square term of age were associated with larger CRVE.

There was a significant interaction between sex and smoking with respect to the CRVE ($P = 0.02$). When analyses were performed separately according to sex, a significantly wider CRVE in smokers than in nonsmokers was detected only in women.

Table 3 demonstrates the differences in mean retinal vessel diameters according to the current smoking status after adjusting for possible confounders. Among all subjects, when modeling with stratum of every 10 cigarettes smoked per day, CRVE was larger in those who smoked 20 or more cigarettes per day by 5.96 μm compared with the eyes of never smokers ($P = 0.01$). Among the females, smokers had larger CRVE values by 9.52 μm (10 to 20 cigarettes per day) and 16.9 μm (more than 20 cigarettes per day), respectively, in comparison with never smokers.

Associations Between Smoking Cessation and the Retinal Vessel Diameters

There was a significant difference in the mean CRVE between the current smokers and subjects with more than 10 years of smoking cessation ($P = 0.003$) when both sexes were combined, whereas there were no significant differences in the mean CRVE between the current smokers and the subjects with fewer than 10 years of smoking cessation ($P = 0.44$) (Table 4).

Although we did not find statistically significant differences in the mean CRAE or CRVE among the past smoking groups in men, there was a significant difference in the mean CRVE

Table 4. Mean Retinal Vessel Caliber Differences According to Current and Past Smoking Status

Smoking Status	*n*	CRAE, µm	Difference Relative to Never Smoker* [95% CI]	*P* Value	*P* Value†	CRVE, µm	Difference Relative to Never Smoker* [95% CI]	*P* Value	*P* Value†
All subjects	1615								
Never smoker	956	128.4	—	—	—	195.8	—	—	—
≥10 years of cessation	336	128.3	−0.11 [−2.20–1.99]	0.93	0.11	195.4	−0.42 [−3.28–2.74]	0.77	**0.003**
<10 years of cessation	156	129.9	1.55 [−1.11–4.22]	0.30	0.81	199.1	3.30 [−0.36–6.97]	0.08	0.44
Current smoker	167	130.3	1.92 [−0.68–4.52]	0.18	—	**200.7**	**4.87 [1.29–8.44]***	**0.008**	—
Test for trend		**0.09**				**0.003**			
Men	598								
Never smoker	84	126.6	—	—	—	197.8	—	—	—
≥10 years of cessation	254	128.1	1.50 [−1.73–4.73]	0.36	0.48	198.8	0.28 [−4.04–4.60]	0.90	0.16
<10 years of cessation	131	129.8	3.17 [−0.39–6.74]	0.08	0.70	201.3	3.55 [−1.22–8.31]	0.15	0.81
Current smoker	129	129.2	2.56 [−1.12–6.25]	0.17	—	200.8	3.02 [−1.88–7.93]	0.23	—
Test for trend		0.11				0.07			
Women	1017								
Never smoker	872	129.0	—	—	—	194.4	—	—	—
≥10 years of cessation	82	126.5	−2.53 [−5.72–0.65]	0.12	**0.02**	191.8	−2.67 [−6.98–1.65]	0.23	**0.0003**
<10 years of cessation	25	128.6	−0.43 [−5.96–5.09]	0.88	0.21	198.3	3.84 [−3.80–11.5]	0.32	0.14
Current smoker	38	133.0	3.98 [−0.46–8.42]	0.08	—	205.3	10.9 [4.64–17.1]*	0.0007	—
Test for trend		0.41				0.007			

* Adjusted for age, sex, city, MAP, WBC, diabetes, BMI, cholesterol, triglycerides, hypertensive medications, and weighted absorbed eye dose.
† *P* values versus current smokers.

between the current smokers and those with more than 10 years of smoking cessation in women ($P = 0.0003$). There was also a statistically significant reduction in the mean CRAE between the current female smokers and the females with more than 10 years of smoking cessation (Table 4).

Discussion

We confirmed a dose-response relationship between smoking and a wider retinal venular caliber in elderly Japanese females. This venodilation associated with smoking appeared to be reversible after 10 or more years of smoking cessation. Our results also showed that female smokers had larger CRVE values than male smokers, and the difference in CRVE between smokers and nonsmokers among females was greater than that observed among males (Tables 2, 3). These findings suggest that there is a sex difference in vessel diameter and a sex difference in small-vessel diameter in response to the effects of smoking. In contrast, the CRAE was not related to the frequency of cigarette smoking or the length of smoking cessation in males in this study.

Previous cross-sectional[8,20] and longitudinal studies with 5-year follow-up[8,20] have reported significant associations between smoking and larger CRVE values,[9] whereas the benefits of quitting tobacco after 5 years of cessation have not been clarified.[21] In this report, we provide additional support for the association between smoking and wider retinal venules by showing a dose-dependent relationship, especially among females. Moreover, the effects of smoking on retinal venular caliber widening may be reversible in females who have stopped smoking for 10 or more years. Given that a larger retinal venular caliber is linked to an increased risk of stroke,[22] it is possible that the effects of smoking on small venular dilation partly account for the effects of smoking on the risk of stroke.

A wider retinal venular caliber is linked to cerebral hypoxia, endothelial dysfunction, hyperglycemia, and inflammation, in contrast to narrower retinal arterioles, which are consistently and strongly related to elevated blood pressure and endothelial dysfunction.[23] Smoking is associated with an increased retinal venular diameter.[8,21] Cigarette smoking not only acts as an inflammatory stimulus, evoking increased levels of C-reactive protein,[24] IL-6,[24] plasma fibrinogen,[24] and WBC, in addition to an increased erythrocyte sedimentation rate,[8] but also functions as a stimulus for endothelial dysfunction.[21] In our study sample, we confirmed that smoking is associated with wider retinal venules, independent of the WBC and C-reactive protein levels (data not shown). Further studies are warranted to fully elucidate all aspects of the effects of smoking on the microcirculation system.

Sex differences in vessel diameter and the reaction of small vessels to smoking are plausible. Our results showed that the vascular effects of smoking in females are more distinctive than those observed in males (Tables 2–4). There is a report supporting a sex difference in CRAE,[25] although the findings are inconclusive. Estradiol/progesterone is known to modulate airway inflammation,[26] lower metabolism in nicotine intake,[27] and induce a higher response to non-nicotine stimuli in females who smoke.[28] These properties of estradiol/progesterone may contribute to an increased susceptibility to larger CRVE values in female smokers. Although the participants were older in age and had undergone menopause, the vascular effects of smoking were distinctively different between the females and males. Alternatively, the difference in CRVE between the current smokers and nonsmokers in women observed in this study could have been due to chance alone, as there were fewer female smokers than male smokers in our study sample.

One of our study strengths is that a well-established computer imaging program was used to measure and summarize the CRAE and CRVE values by a trained grader who was blinded to the participant characteristics. In addition, we had several sources of information regarding the past smoking histories of the subjects, such as interview/mail surveys conducted in the 1960s and/or 1970s in addition to the detailed smoking habit data obtained in the biennial examinations conducted since approximately 1990. These

data enabled us to investigate the duration of smoking cessation more accurately than an ordinary cross-sectional analysis, which relies on data obtained at one specific point in time.

There are limitations associated with our study. We cannot exclude the possibility that the associations observed in this study are due to residual confounders (e.g., unmeasured endothelial function, the use of hormone replacement therapy) and selection bias due to the exclusion of participants with either no fundus photos or inadequate quality photographs for retinal vessel caliber assessment. We also cannot exclude the possibility that the associations found were due to chance because the number of smokers among females was small. Finally, our results may not be directly applicable to the general population, as atomic bomb survivors exposed to radiation were included in this analysis. A mortality bias also should be noted. The number of participants in the AHS has decreased over the past 50 years. Therefore, individuals with a high level of radiation exposure may have died before being included in this study. Furthermore, our results may not be applicable to the general population.

In summary, we confirmed the presence of an association between smoking and the CRVE and further expanded current understanding by showing that there is a dose-dependent association between an increased number of cigarettes smoked per day and a widening CRVE. Most importantly, we found that venodilation associated with smoking appears to be reversible after 10 or more years of smoking cessation. Given that retinal venular dilation has been linked to an increased risk of stroke, our findings reinforce the importance of smoking cessation in maintaining vascular health among older people.

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