# Retinal Vessels and Diseases in the Elderly
# The Rotterdam Study

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# Retinal Vessels and Diseases in the Elderly
# The Rotterdam Study

Retinavaten en ouderdomsziekten
Het ERGO-onderzoek

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. S.W.J. Lamberts
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Mohammad Kamran Ikram
geboren te Rotterdam

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| Promotoren: | Prof.dr. P.T.V.M. de Jong<br>Prof.dr. M.M.B. Breteler |
| Overige leden: | Prof.dr. C.I. de Zeeuw<br>Prof.dr. P.J. Koudstaal<br>Prof.dr. C.J.J. Avezaat |

GOD gave me this life;
my father trained it,
my mother raised it.

*For my parents*

## PUBLICATIONS AND MANUSCRIPTS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

**Chapter 2**
M.K. Ikram, F.J. de Jong, J.R. Vingerling, J.C.M. Witteman, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2004; 45: 2129-2134

**Chapter 3.1**
R. van Leeuwen, M.K. Ikram, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong. Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2003; 44: 3771-3777

**Chapter 3.2**
M.K. Ikram, R. van Leeuwen, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong. Retinal vessel diameters and the risk of incident age-related macular disease: The Rotterdam Study. *Ophthalmology* 2005; 112: 548-552

**Chapter 3.3**
M.K. Ikram, R. van Leeuwen, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong. Relationship between refraction and prevalent as well as incident age-related maculopathy: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2003; 44: 3778-3782

**Chapter 3.4**
M.K. Ikram, S. de Voogd, R.C.W. Wolfs, A. Hofman, M.M.B. Breteler, L.D. Hubbard, P.T.V.M. de Jong. Retinal vessel diameters and incident open-angle glaucoma and optic disc changes: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2005; 46: 1182-1187

**Chapter 4.1**
M.K. Ikram, F.J. de Jong, M. Bos, J.R. Vingerling, A. Hofman, P.J. Koudstaal, P.T.V.M. de Jong, M.M.B. Breteler. Retinal vessel diameters and risk of stroke: The Rotterdam Study. *Submitted*

**Chapter 4.2**
M.K. Ikram, F.J. de Jong, E.J. van Dijk, N.D. Prins, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Retinal vessel diameters and cerebral small vessel disease: The Rotterdam Scan Study. *Submitted*

**Chapter 4.3**
F.J. de Jong, M.K. Ikram, P.J. Koudstaal, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong, M.M.B. Breteler. Retinal arteriolar and venular diameters and risk of dementia: The Rotterdam Study. *Submitted*

**Chapter 4.4**
M.K. Ikram, J.R. Vingerling, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Aging macular disease in relation to Alzheimer's disease and brain atrophy on MRI. *Submitted*

**Chapter 5.1**
M.K. Ikram, J.R. Vingerling, A. Hofman, J.C.M. Witteman, M.M.B. Breteler, P.T.V.M. de Jong. Retinal vessel diameters and risk of hypertension: The Rotterdam Study. *Submitted*

**Chapter 5.2**
M.K. Ikram, J.A.M.J.L. Janssen, A.M.E. Roos, I. Rietveld, A. Hofman, C.M.van Duijn, P.T.V.M. de Jong. Retinal vessel diameters and risk of diabetes mellitus: The Rotterdam Study. *Submitted*

**Chapter 5.3**
M.K. Ikram, J. Heeringa, J.R. Vingerling, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Retinal vessel diameters and all-cause mortality: The Rotterdam Study. *To be submitted*

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# *Part I*

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# *Prologue*

# Chapter 1

## A Historical Perspective

*"I examined, using myopic spectacles, the eye of a dog, placing a candle behind the animal as the light source. Lo and behold! Each time I examined the eye from one direction in particular, a glimmering light appeared. Immediately repeating the same experiment on man, the identical phenomenon occurred, namely, the entire pupil glowed with a vivid orange color[1]…… If clinicians do not reject, look down on, or shudder at the anxious inquiries of physiologists, they will find this technique of considerable value[2]……"*

Thus wrote the Czech physiologist, Johannes Evangelista Purkinje (figure 1), in his thesis *Commentatio de examine physiologico organi visus et systematis cutanei*, which was published in 1823 and was based on his early experimental work at the University of Prague. With the use of his makeshift ophthalmoscope he became the first person to examine the living human retina. However, Purkinje's pioneering work went virtually unnoticed.

It took another three decades before the German physician-scientist Hermann von Helmholtz (figure 1) in 1851 devised the first ophthalmoscope based on the same principles as were used by Purkinje.[3] Helmholtz created his ophthalmoscope without knowledge of Purkinje's work. Unlike his predecessor, Helmholtz publicized his findings by personally visiting German universities to introduce his invention to the influential academic community. Therefore, Helmholtz is usually accredited with the invention of the ophthalmoscope.

The retina as seen through the ophthalmoscope opened a window on the living human microcirculation in a natural and non-invasive way. The potential of retinal vessel signs to serve as markers of cardiovascular and cerebrovascular disease was soon recognized by the Scottish physician Robert

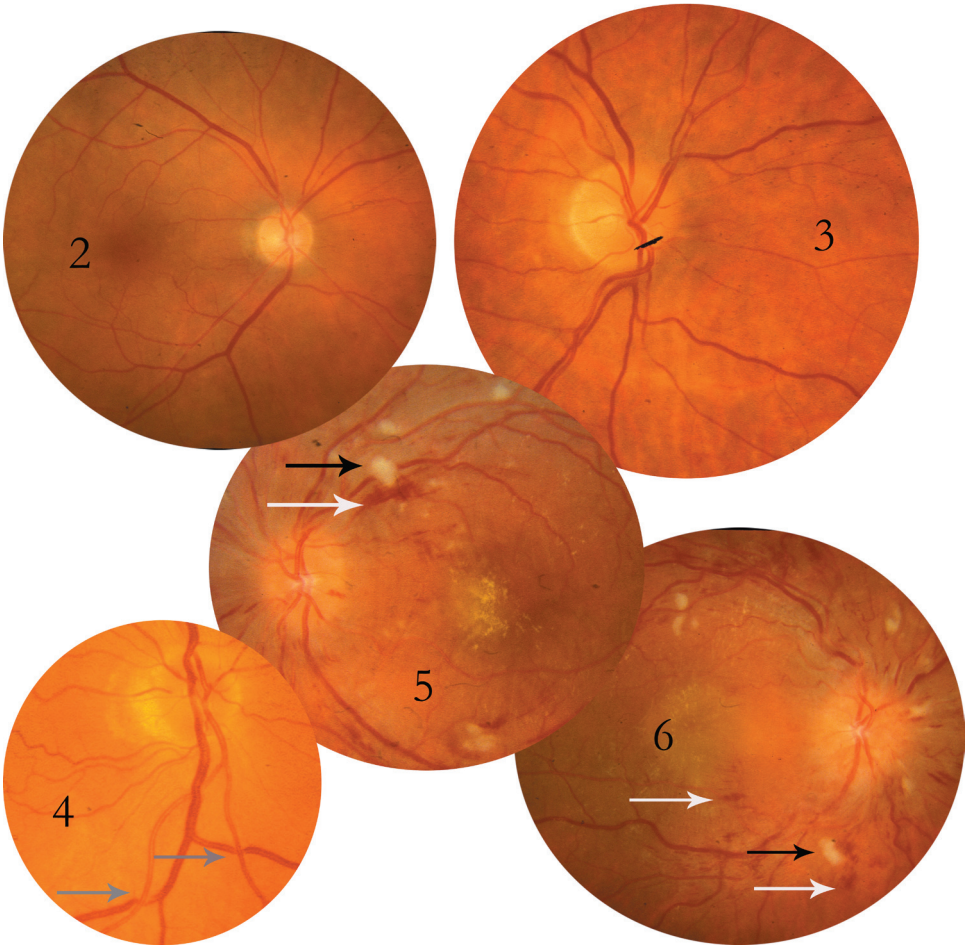
**Figure 1**. Left: Johannes Purkinje (1787-1869); Center: Hermann von Helmholtz (1821-1894); Right: Robert Marcus Gunn (1850-1909)

Marcus Gunn (figure 1), who in 1898 presented a series of observations he made on the retinal vessels in patients with cerebrovascular disease.[4, 5] The signs he described were generalized arteriolar narrowing, arterio-venous nicking, cotton-wool spots, intra-retinal hemorrhages and papilledema. In 1939, Keith, Wagener and Barker made the first attempt to relate these fundus signs to survival of patients with hypertension (table 1; figures 2 to 6). They divided 219 hypertensive patients into four groups according to four grades of hypertensive retinopathy. The 3-year survival of persons with grade I was 70%, compared to only 6% in those with grade IV.[6] Subsequently, other additional classification schemes for hypertensive retinopathy (table 1) were proposed and their relationship with cardiovascular diseases and mortality was described.[6-8]

Although these classifications provided a better understanding of hypertensive retinopathy, application of these systems to a clinical setting proved difficult due to several reasons. The natural sequence of these grades was not clear; for example in some cases grade III was observed to occur before grade II. Furthermore, papilledema (grade IV) can develop in patients with malignant hypertension without showing any signs of earlier grades.[9] Most of the pioneering work was done in clinic-based populations with hypertension in a period with limited possibilities for treatment.[5] The more severe abnormalities were shown to be relatively uncommon in populations that were examined a few decades later, perhaps due to better control of blood pressures.[10, 11] Finally, the detection of retinal vessel signs with ophthalmoscopy has been demonstrated to be subjective and less reliable.[4, 12, 13] In particular, generalized retinal arteriolar narrowing, which is one of the earliest signs, proved to be difficult to quantify in an objective way.[4, 14]

**Table 1**. Summary of different classification systems of retinal vessel signs of atherosclerosis and hypertension

| Grade | Retinal vessel signs |
|---|---|
| Keith, Wagner and Barker (1939) | |
| I | Mild to moderate narrowing or sclerosis of the arterioles |
| II | Moderate to marked arteriolar sclerosis; arterio-venous nicking; generalized and/or focal arteriolar narrowing |
| III | Marked arteriolar narrowing and focal constriction; retinal edema; cotton-wool spots; hemorrhages |
| IV | Grade III plus papilledema |
| Wagener, Clay and Gipner (1947) | |
| 1. Neurogenic | Mild generalized arteriolar narrowing |
| 2. Acute (angiospastic) | Moderate generalized arteriolar narrowing; focal constriction; retinal edema; cotton-wool spots; hemorrhages |
| 3. Chronic non-progressive | Marked generalized arteriolar narrowing |
| 4. Chronic progressive | Generalized and focal arteriolar narrowing and sclerosis |
| 5. Malignant | Papilledema |
| Scheie (1953) | |
| I | Barely detectable arteriolar narrowing or light reflex changes |
| II | Obvious arteriolar narrowing with focal irregularities or increased light reflex changes |
| III | Grade II + hemorrhages and/or exudates or copper-wire arterioles |
| IV | Grade III + papilledema or silver-wire arterioles |
| Leishman (1957) | |
| 1. Involutional sclerosis | Arterioles: straight and narrow; no arterio-venous nicking; general picture of an aging fundus |
| 2. Involutional sclerosis with hypertension | Arteriolar segment towards disc dilated; distal arteriolar segment narrow |
| 3. Involutional sclerosis with advanced hypertension | Major arterioles dilated; arterio-venous crossing changes |
| 4. Early hypertension in youthful vessels | Generalized arteriolar narrowing |
| 5. Fulminating hypertension | Arteriolar narrowing; papilledema and retinal edema, hemorrhage, arterio-venous nicking |
| 6. Severe hypertension with relative sclerosis | Grades 1 to 4 |




**Figures 2 to 6**. Fundus photographs showing different retinal vessel signs: generalized arteriolar narrowing (2 and 3); arterio-venous nicking (grey arrows in 4); cotton-wool spots (black arrows in 5 and 6); hemorrhages (white arrows in 5 and 6); and papilledema (6).

Since the 1970s semi-objective methods based on retinal photography or slide projection systems have been developed.[5] These methods relied on obtaining a ratio between arteriolar and venular widths as an index of generalized arteriolar narrowing. In 1974, Parr and Spears developed a technique for evaluation of generalized arteriolar narrowing.[15, 16] They measured all retinal arteriolar blood column diameters in an area between half a disc-diameter and one disc-diameter from the optic disk margin. This region was selected because its vessels unequivocally are arterioles instead of arteries.[5, 14] Furthermore, in this region there was less overlap between the vessels than near or on the optic disc making the measurements more

reliable. The measured arteriolar diameters were converted into one sum value, namely the "central retinal artery equivalent".[14-16] Hubbard et al. later extended Parr's method to venules and summarized these as "central retinal vein equivalent".[14] Using this technique, they developed a semi-automated system to evaluate more reliably and accurately retinal vessel diameters from high-resolution digitized fundus photographs (figure 7).[14] As a marker of generalized retinal arteriolar narrowing they also proposed to use the ratio of the arteriolar to venular sum diameter values, because this ratio levels out artifacts caused by magnification differences due to refractive errors of the eye.[5, 14] Subsequently, it was shown that a smaller arteriolar-to-venular ratio (AVR) might provide information on predicting cardiovascular and cerebrovascular diseases independently of known risk factors.[14, 17-19] Compared to other retinal signs like hemorrhages, focal arteriolar narrowing or arterio-venous nicking, the AVR was the more reliable and commonly used parameter of vascular damage. It remained unclear, however, in which way the separate arteriolar or venular diameters contributed to this ratio and what kind of vascular pathology this ratio precisely reflects. Furthermore, it was unknown whether the associations with cardiovascular diseases were due to

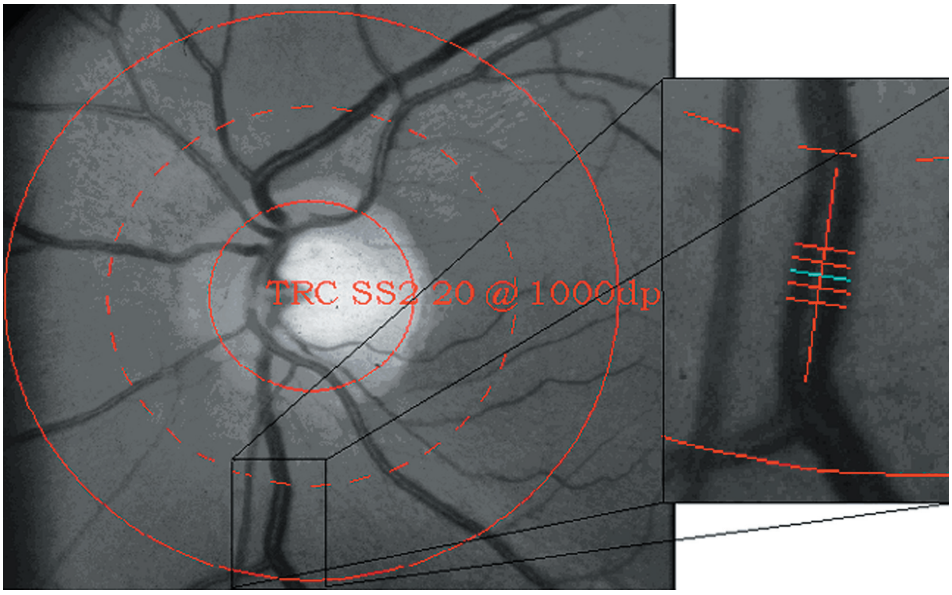



**Figure 7**. The grid is composed of three circles concentric with the optic disc. The innermost circle represents the average optic disc area. The diameters of all vessels (arterioles: lighter and venules: darker) that cross the area bounded by the dotted circle (a half disc diameter) and outermost circle (one disk diameter) were measured.

generalized arteriolar narrowing, venular dilatation or a combination of both. Thus far, data were lacking with respect to the role of the separate arteriolar and venular diameters in relation to diseases in the elderly.

## Scope of the Thesis

I hypothesized that the described associations of the AVR might be based on venular dilatation rather than arteriolar narrowing. Thus, the main objective of the work presented in this thesis was to study the association between separate arteriolar and venular diameters and the risk of ophthalmological, neurological and systemic diseases. We performed our retinal vessel measurements, using the semi-automated system developed by Hubbard et al., within the setting of the Rotterdam Study. The Rotterdam Study is a large on-going prospective, population-based cohort study among 7983 persons (response rate 78%) aged 55 years or older, which focuses on cardiovascular, endocrine, neurological and ophthalmological diseases in the elderly.[20] Baseline interview and examinations were performed from 1990 to 1993.

Because it was unclear what kind of vascular pathology the AVR reflects and how the separate arteriolar and venular diameters may contribute to it, chapter 2 describes a study in which we examined associations between retinal arteriolar or venular diameters and their AVR, and markers of subclinical cardiovascular disease.

In chapters 3.1 to 3.4, I focus on ophthalmological diseases. The role of vascular factors in aging macular disease* (AMD) was assessed by studying classical risk factors such as blood pressure and markers of atherosclerosis (chapter 3.1). To further explore its vascular pathogenesis, retinal vessel diameters were related to the risk of AMD (chapter 3.2). A prominent role in the etiology of AMD has been attributed to a disturbed choroidal circulation.[21, 22] That made us study in chapter 3.3 if hyperopia, that may lead to impaired choroidal circulation, presumably due to increased scleral rigidity, is associated with AMD. Chapter 3.4 addresses the issue whether

* In this thesis several terms are used to denote the same progressive and degenerative disease in elderly persons affecting the macula. In a few publications we used the traditional term age-related maculopathy (ARM) to denote this disease. However, due to evolution in thinking and terminology, a new term (age-related macular disease) was coined by Bird [Eye 2003; 17: 457-466]. More recently, we proposed to use the term "aging macular disease". Hence, in this thesis the reader will come across all three terms.

retinal vessels may play a causative role in the pathophysiology of open-angle glaucoma.

Apart from the role of retinal vessels in ocular diseases, these vessels have also been suggested to provide information on vascular pathology of the brain, because they share many features with the cerebral vessels such as blood-tissue barrier, auto-regulation and embryological origin.[23] The next four chapters are, therefore, devoted to cerebrovascular diseases. These successive chapters describe the role of retinal arteriolar and venular diameters in determining the risk of stroke (chapter 4.1), cerebral small vessel disease (chapter 4.2) and dementia (chapter 4.3), including its major subtype Alzheimer's disease. Because extra-cellular deposits characterize both AMD and Alzheimer's disease,[24, 25] it has been suggested that AMD may be an ocular manifestation of Alzheimer's disease. The possible link between AMD and Alzheimer's disease is given in chapter 4.4.

Chapter 5 explores the associations between retinal arteriolar and venular diameters and the risk of systemic diseases as hypertension (chapter 5.1) and diabetes mellitus (chapter 5.2). Whether retinal vessel diameters predict all-cause mortality is shown in chapter 5.3.

Finally, in chapter 6, the main findings, methodological considerations, suggestions for future (epidemiological) research, and final conclusions are discussed.

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# *Part II*

# *Retinal Vessel Measurements*

# Chapter 2

# Are Retinal Arteriolar or Venular Diameters Associated with Markers for Cardiovascular Disorders? The Rotterdam Study


## ABSTRACT

***Purpose*** *– Lower retinal arteriolar-to-venular ratio (AVR) has been suggested to reflect generalized arteriolar narrowing and to predict the risk of cardiovascular diseases. The contribution of the separate arteriolar and venular diameters to this AVR is unknown. Thus associations between retinal arteriolar and venular diameters, and the AVR on the one hand and blood pressure, atherosclerosis, inflammation markers and cholesterol levels on the other were examined in the Rotterdam Study.*

***Methods*** *– In this cross-sectional population-based study, for one eye of each subject (≥ 55 years; n = 5674) retinal arteriolar and venular diameters (in μm) of the blood columns were summed on digitized images. At baseline blood pressures, cholesterol levels, and markers of atherosclerosis and inflammation were also measured.*

***Results*** *– With increasing blood and pulse pressures retinal arteriolar and venular diameters, and the AVR decreased significantly and linearly. Lower arteriolar diameters were associated with increased carotid intima-media thickness. Larger venular diameters were associated with higher carotid plaque-score, more aortic calcifications, lower ankle-arm index, higher leukocyte count, higher erythrocyte sedimentation rate, higher total serum cholesterol, lower HDL, higher waist-to-hip ratio and smoking. A lower AVR was related to increased carotid intima-media thickness, higher carotid plaque-score, higher leukocyte count, lower HDL, higher body mass index, higher waist-to-hip ratio and smoking.*

***Conclusions*** *– Because larger venular diameters are associated with atherosclerosis, inflammation and cholesterol levels, the AVR does not depend only on generalized arteriolar narrowing due to the association between smaller arteriolar diameters and higher blood pressures. These data indicate that retinal venular diameters are variable and may play their own independent role in predicting cardiovascular disorders.*


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Structural changes in the retinal vasculature have long been recognized as an important predictor of systemic hypertensive damage and life prognosis.[1,2] Different classifications mainly depended on qualitative assessment of retinal vessels using ophthalmoscopy, which has a poor reproducibility and gauges arteriolar diameters against the venular ones.[3]

Recently, in the Atherosclerosis Risk in Communities (ARIC) Study a semi-automated system was developed to measure the vessel diameters on fundus photographs.[4-6] The authors attributed a lower arteriolar-to-venular ratio (AVR) to generalized arteriolar narrowing and suggested that this ratio might provide information in predicting incident cardiovascular diseases independently of known cardiovascular risk factors.[6-14] Compared to other retinal signs like hemorrhages, focal arteriolar narrowing or arterio-venous nicking, the AVR was the more reliable and commonly used parameter of vascular damage.[6,8-11,15]

It remains unclear, however, what exactly the separate arteriolar and venular diameters contributed to the AVR and what kind of vascular pathology this ratio precisely reflects. Limited data have been published on the association between AVR or retinal vessel diameters and blood pressure.[6,11-13] Associations concerning AVR and atherosclerosis were inconclusive.[15,16]

Currently, no data are available on the specific relationship between arteriolar or venular diameters and atherosclerosis, inflammation markers or cholesterol levels. We therefore investigated in a population-based setting these cross-sectional associations.

## METHODS AND MATERIALS

### Population

The present study was performed as part of the Rotterdam Study, a population-based, cohort study on chronic and disabling diseases in the elderly. A total of 7983 subjects aged 55 years and older living in a district of Rotterdam agreed to participate in the study. Information on the baseline study population has appeared in previous reports.[17,18] Because the ophthalmic part became operational after the screening of participants had started, a smaller number (n = 6780) participated in the ophthalmic examination.[19] The study was conducted according to the tenets of the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline interviews and examinations were performed from 1990 to mid-1993.

### Retinal vessel measurements

After the eye examination at baseline simultaneous stereoscopic fundus color transparencies centered on the optic disc (pharmacological mydriasis, $20^0$ field, Topcon Optical Company, Tokyo, Japan) were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan).[18] Per subject the image with the best quality (left or right eye) was analyzed with a semi-automated system (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison) by four trained graders masked for the endpoints.[4-6] We used the improved Parr-Hubbard formula to compute the summary vessel measures. Because each eye has a different magnification due to its optical system (lens, cornea) we additionally adjusted this summary vessel measure for the refraction and corneal curvature using Littmann's formula to obtain absolute measures.[20,21] On one eye of each subject one sum value was calculated for the arteriolar blood column diameter and one for the venular (in μm), the AVR being the ratio of these. In a random sub-sample of 100 subjects we found no differences between the right and left eyes for the arteriolar and venular diameters.

Quality control sessions with a random sub-sample of 40 transparencies gave the following Pearson's correlation coefficients for inter-grader agreement 0.67-0.80 (arteriolar diameter), 0.91-0.94 (venular diameter) and 0.75-0.84 (AVR). For intra-grader agreement these were 0.69-0.88, 0.90-0.95 and 0.72-0.90, respectively.

**Blood pressure, atherosclerosis, inflammation and cholesterol**

Blood pressures, ankle-arm index, intima-media thickness, carotid artery plaques and aortic atherosclerosis were measured as described previously.[22-24]

Briefly, intima-media thickness of the common carotid artery was assessed by ultra-sonography, using a 7.5-MHz linear-array transducer (ATL Ultra-Mark IV). For the current analyses, the average of the mean anterior and posterior intima-media thickness of both the left and the right common carotid artery was used. Atherosclerotic plaques, assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides, were defined as focal thickening of the vessel wall (of at least 1.5 times the average intima-media thickness) relative to adjacent segments with or without calcified components. The carotid plaque-score (range:0-6) reflects the number of these locations with plaques. Aortic atherosclerosis defined as calcified deposits in the abdominal aorta on lateral radiographic films of the lumbar spine, was considered present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). The extent of calcification was scored according to the length of the involved area (0, 0.5 to <1, 1 to <2.5, 2.5 to <5, 5 to <10, and ≥10 cm). All readers and technicians were masked for all clinical information.

Leukocyte count was assessed in citrate plasma using a Coulter Counter T540® (Coulter electronics, Luton, England). Blood was drawn directly into VACUTAINER® tubes and erythrocyte sedimentation rate was read after 60 minutes. Non-fasting serum total cholesterol and high-density lipoprotein (HDL) concentrations were determined by an automated enzymatic procedure.[25] Information on smoking (categorized as current, former or never) and medication use was obtained during the home interview. Alcohol consumption was assessed as part of a dietary interview.[26]

**Study sample**

Of the 6780 participants in the ophthalmic part, 6436 persons underwent optic disc photography. From these 762 subjects were excluded because they had ungradable fundus transparencies on both eyes, resulting

in a cohort of 5674 subjects. Measurements on blood pressure were missing in 91 participants, ankle-arm index in 547, intima-media thickness in 918, carotid plaque-score in 970, aortic calcifications in 1510, leukocyte count in 394, erythrocyte sedimentation rate in 1543, total cholesterol in 44, HDL cholesterol in 53, body mass index in 72, waist-to-hip ratio in 350, smoking

**Table 1**. Baseline Characteristics presented as unadjusted means (SD) or percentages

| | **Gradable*** | **Ungradable** | **Adjusted differences† (95% CI)** |
|---|---|---|---|
| Number (n) | 5674 | 762 | |
| Age (years) | 68.0 (8.2) | 75.7 (10.1) | **7.8 (7.1; 8.4)§** |
| Gender (% female) | 59.0 | 63.0 | 0.1 (-3.8; 4.0) |
| Institutionalized (%) | 3.6 | 22.0 | **9.9 (8.3; 11.6)** |
| Diabetes Mellitus (%) | 10.0 | 14.0 | 0.9 (-1.4; 3.3) |
| Systolic blood pressure (mmHg) | 138.5 (22.1) | 145.2 (24.0) | 1.5 (-0.2; 3.3) |
| Diastolic blood pressure (mmHg) | 73.7 (11.4) | 74.2 (12.8) | **1.4 (0.5; 2.3)** |
| Pulse pressure (mmHg) | 64.8 (17.7) | 71.0 (19.7) | 0.1 (-1.2; 1.4) |
| Ankle-arm index | 1.07 (0.22) | 0.97 (0.28) | **-0.03 (-0.05; -0.02)** |
| Carotid Intima-media thickness (mm) | 0.79 (0.15) | 0.86 (0.19) | **0.02 (0.01; 0.03)** |
| Aorta calcification ≥ 5 cm (%) | 11.1 | 17.6 | 2.1 (-1.2; 5.4) |
| Carotid plaques ≥ 4 (%) | 16.1 | 24.0 | 1.5 (-1.7; 4.7) |
| Leukocyte count ($10^9$/L) | 6.7 (1.9) | 6.8 (3.0) | 1.1 (-0.1; 0.2) |
| Erythrocyte sedimentation rate | 12.9 (10.7) | 16.2 (14.4) | **1.3 (0.2; 2.3)** |
| Total serum cholesterol (mmol/l) | 6.63 (1.21) | 6.53 (1.30) | 0.01 (-0.1; 0.1) |
| Serum HDL cholesterol (mmol/l) | 1.35 (0.36) | 1.34 (0.36) | 0.00 (-0.02; 0.03) |
| Body mass index (kg/$m^2$) | 26.3 (3.68) | 26.0 (3.69) | **-0.44 (-0.73; -0.14)** |
| Waist-to-hip ratio | 0.90 (0.09) | 0.91 (0.09) | 0.00 (-0.01; 0.01) |
| Smoking (%) | | | |
| Current | 23.6 | 18.7 | 0.4 (-2.9; 3.7) |
| Former | 42.9 | 36.8 | -3.7 (-7.4; 1.9) |
| Alcohol consumption (%) | | | |
| ≤ 10 gram/day‡ | 45.0 | 41.9 | -3.3 (-8.1; 1.5) |
| > 10 - ≤ 20 gram/day | 15.4 | 13.0 | -1.3 (-4.8; 2.2) |
| > 20 gram/day | 19.5 | 19.3 | 1.0 (-2.7; 4.7) |

* Subjects with a gradable fundus transparency on at least one eye
† Age and gender adjusted if applicable
‡ 10 grams is on average equal to 1 drink
§ Significant ($p < 0.05$) data in bold

**Table 2**. Age and gender adjusted difference (95% CI) in retinal vessel diameters with increasing blood pressure levels both per SD and per 10-mmHg and with increasing severity of sub-clinical atherosclerosis.

| | N | Arteriolar diameter (μm) | Venular diameter (μm) | Arteriolar-to-venular ratio |
|---|---|---|---|---|
| **Blood pressure** | | | | |
| Systolic Blood Pressure: | | | | |
| Per SD increase | 5583 | **-2.4 (-2.8; -2.0)*** | **-1.0 (-1.6; -0.5)** | **-0.008 (-0.009; -0.006)** |
| Per 10-mmHg increase | | **-1.1 (-1.3; -0.9)** | **-0.5 (-0.7; -0.2)** | **-0.0035 (-0.004; -0.003)** |
| Diastolic Blood Pressure: | | | | |
| Per SD increase | 5583 | **-2.4 (-2.8; -2.1)** | **-0.8 (-1.3; -0.2)** | **-0.009 (-0.010; -0.007)** |
| Per 10-mmHg increase | | **-2.1 (-2.5; -1.8)** | **-0.7 (-1.1; -0.2)** | **-0.008 (-0.009; -0.006)** |
| Pulse Pressure: | | | | |
| Per SD increase | 5583 | **-1.5 (-1.9; -1.1)** | **-0.8 (-1.4; -0.3)** | **-0.004 (-0.006; -0.003)** |
| Per 10-mmHg increase | | **-0.8 (-1.1; -0.6)** | **-0.5 (-0.8; -0.1)** | **-0.002 (-0.003; -0.001)** |
| **Markers of sub-clinical atherosclerosis** | | | | |
| Carotid Intima-media thickness: | | | | |
| Per SD increase | 4756 | **-0.9 (-1.4; -0.5)** | -0.2 (-0.8; 0.5) | **-0.004 (-0.005; -0.002)** |
| Carotid plaque-score: | | | | |
| Per plaque increase | 4704 | -0.2 (-0.5; 0.1) | **0.4 (0.1; 0.8)** | **-0.002 (-0.003; -0.001)** |
| Ankle-arm index: | | | | |
| Per SD decrease | 5127 | 0.4 (0.0; 0.8) | **1.3 (0.7; 1.9)** | **-0.002 (-0.0004; -0.004)** |
| Aorta calcification: | | | | |
| Per category increase | 4164 | 0.03 (-0.3; 0.4) | 0.4 (-0.1; 0.8) | -0.001 (-0.002; 0.001) |

* Significant ($p < 0.05$) data in bold

in 65 and alcohol consumption in 884. Restricted availability of technicians was the main reason for missing data, which was independent of any subject characteristics.

**Statistical analysis**

Analysis of covariance (ANCOVA) was used to compare subjects with gradable and ungradable transparencies and to analyze whether associations were linear. If so, multiple linear regression models were used to assess these cross-sectional relationships. We analyzed blood pressures both per standard deviation (SD) and per 10-mmHg increase. The former was taken to compare the effect size between systolic and diastolic blood pressures, the latter to compare our results to those of other studies.

All analyses were repeated after stratifying on subjects with diseases known to affect venular width such as diabetes mellitus and hypertension. We used SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

Table 1 shows the baseline characteristics of subjects with gradable and ungradable transparencies. The means were: arteriolar diameter 146.9μm (range: 92.2-235.7; SD: 14.4), venular diameter 222.0μm (range: 135.1-313.6; SD: 20.9) and AVR 0.66 (range: 0.48-1.02; SD: 0.06).

**Blood pressures and retinal vessel diameters**

With increasing blood and pulse pressures the arteriolar diameters decreased linearly (table 2). The figure shows that the relationship with the arteriolar diameters was strongest in the youngest age category (per SD increase in systolic blood pressure: -3.8μm (95% CI: -4.7; -2.8)) and became non-significant above age 80 years. With increasing blood and pulse pressures the venular diameters showed a small decrease, and stratified on age there seemed to be no clear trend.

With increasing blood and pulse pressures the AVR decreased linearly (table 2). The relationship with AVR was strongest in the age category 55-60 years (Fig.); per SD increase in systolic blood pressure AVR decreased by 0.016 (95% CI: 0.012; 0.019). Diastolic blood pressure showed the same trends; hence only systolic blood pressure is presented in the figure.

**Atherosclerosis and retinal vessel diameters**

The arteriolar diameters did not show an association with the different

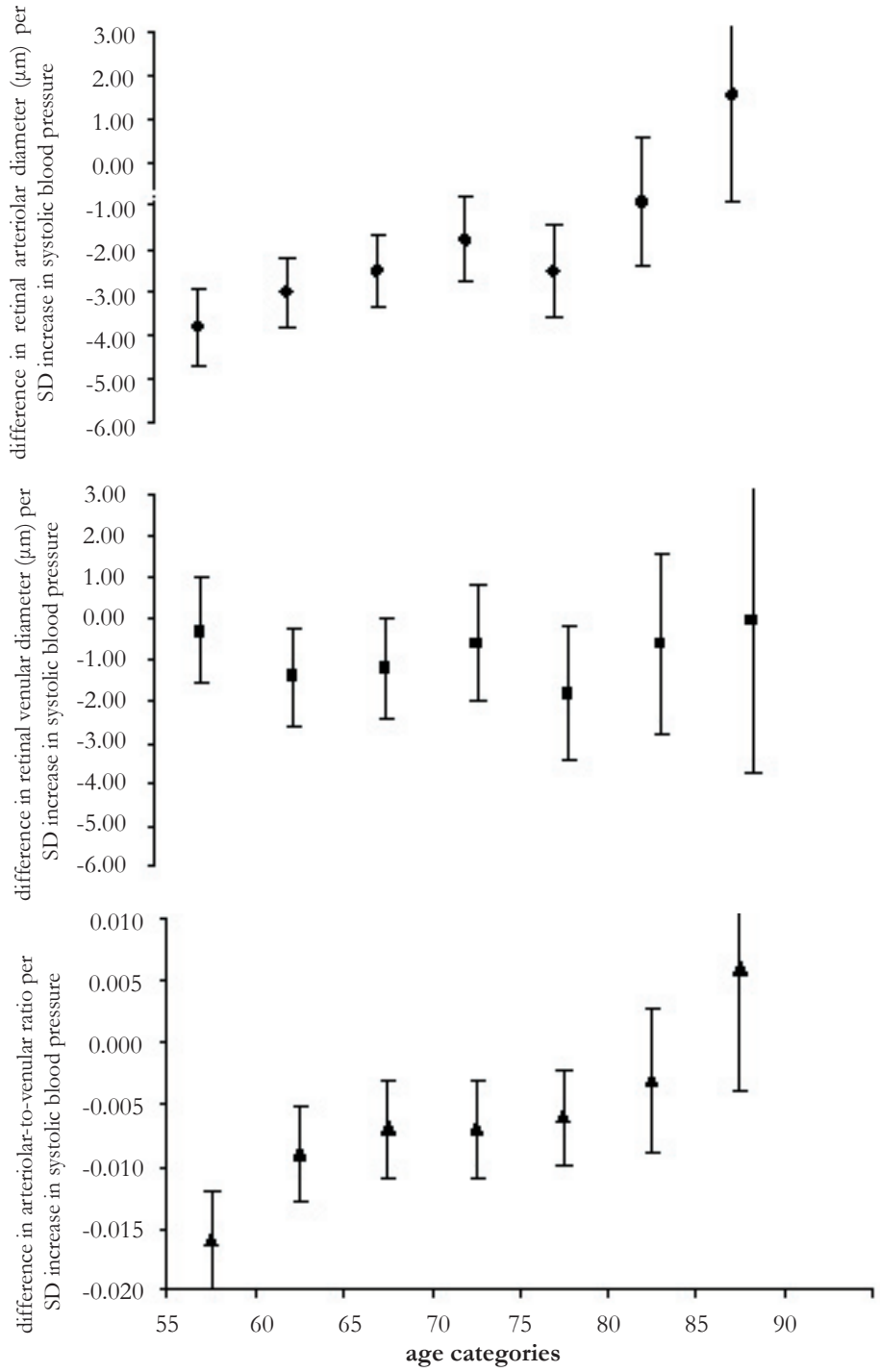


**Figure**. The relationship between blood pressure and retinal vessel measurements per five-years age categories (age category 90-95 years has been excluded due to low numbers n = 40))

markers of atherosclerosis except intima-media thickness. After additional adjustment for blood pressure, this association became weaker (per SD increase: -0.6μm (95% CI: -1.0; -0.1)).

The venular diameters were linearly related to several markers of atherosclerosis. Larger venular diameters were associated with a lower ankle-arm index, higher carotid plaque-score, more aortic calcifications (borderline) and a non-significantly increased intima-media thickness. Additional adjustment for blood pressure did not alter these associations. Stratification on age did not show a trend between atherosclerosis and retinal venular diameters.

A lower AVR was significantly related to a lower ankle-arm index, increased intima-media thickness and higher carotid plaque-score, but not to aortic calcifications (table 2). After additional adjustment for blood pressure, AVR was still associated with intima-media thickness (per SD increase: -0.002; 95% CI: -0.004; -0.001) and carotid plaque-score (per plaque increase: -0.002; 95% CI: -0.003; -0.001).

**Inflammation markers, cholesterol levels and retinal vessel diameters**

Higher leukocyte count, higher erythrocyte sedimentation rate, lower serum HDL levels, higher total serum cholesterol, higher waist-to-hip ratio and smoking were related to larger venular diameters and to a lesser extent to larger arteriolar diameters thus resulting in a lower AVR (table 3).

These analyses were repeated using models in which the cardiovascular variables were categorized. Because all relationships were linear in these analyses, only the linear models are presented. Stratification on subjects with and without hypertension did not significantly alter any of the above-mentioned results. The results were again the same after stratification on diabetes mellitus. However, in subjects with diabetes mellitus the point estimates for these relationships were less stable with wider confidence intervals, because only 10% of the population had diabetes mellitus at baseline.

## DISCUSSION

These results show that higher blood and pulse pressures were related to lower arteriolar diameters. More atherosclerosis as measured by the ankle-arm index, aortic calcifications and carotid plaque-score, higher leukocyte count, higher erythrocyte sedimentation rate, higher total cholesterol, lower HDL levels, higher waist-to-hip ratio or smoking were related to larger venular diameters, the overall effect being a lower AVR.

**Table 3.** Age and gender adjusted difference (95% CI) in retinal vessel diameters with increasing levels of inflammation, cholesterol, smoking and alcohol consumption.

| | N | Arteriolar diameter (µm) | Venular diameter (µm) | Arteriolar-to-venular ratio |
|---|---|---|---|---|
| Leukocyte count: per SD increase | 5280 | **1.1 (0.8; 1.5)*** | **2.9 (2.4; 3.5)** | **-0.003 (-0.005; -0.002)** |
| Erythrocyte sedimentation rate: per SD increase | 4131 | **0.7 (0.3; 1.2)** | **0.8 (0.1; 1.5)** | 0.001 (-0.001; 0.003) |
| Total serum cholesterol: per SD increase | 5630 | 0.2 (-0.2; 0.6) | 0.4 (-0.1; 1.0) | -0.0002 (-0.002; 0.001) |
| Serum HDL cholesterol: per SD increase | 5621 | **-0.5 (-0.9; -0.1)** | **-1.2 (-1.8; -0.7)** | **0.001 (0.000; 0.003)** |
| Body mass index: per SD increase | 5602 | **-0.7 (-1.1; -0.3)** | 0.1 (-0.4; 0.7) | **-0.004 (-0.005; -0.002)** |
| Waist-to-hip ratio: per SD increase | 5324 | -0.2 (-0.6; 0.3) | **1.1 (0.4; 1.7)** | **-0.004 (-0.006; -0.002)** |
| Smoking: | | | | |
| Current vs. non-smokers (n=1324 vs. n=1877) | | **5.0 (3.9; 6.0)** | **10.0 (8.3; 11.4)** | **-0.007 (-0.011; -0.003)** |
| Former vs. non-smokers (n=2408 vs. n=1877) | | **1.3 (0.4; 2.3)** | **2.5 (1.2; 3.9)** | -0.002 (-0.006; 0.002) |
| Alcohol consumption: | | | | |
| ≤ 10 gram/day vs. non-drinkers (n=2158 vs. n=965) | | 0.6 (-0.5; 1.7) | 0.6 (-0.9; 2.2) | 0.001 (-0.004; 0.005) |
| > 10 - ≤ 20 gram/day vs. non-drinkers (n=737 vs. n=965) | | -1.0 (-2.4; 0.4) | -0.9 (-2.9; 1.1) | -0.002 (-0.008; 0.003) |
| > 20 gram/day vs. non-drinkers (n=930 vs. n=965) | | -1.1 (-2.4; 0.3) | 1.5 (-0.4; 3.4) | **-0.010 (-0.015; -0.004)** |

* Significant ($p < 0.05$) data in bold

For proper interpretation some methodological issues warrant consideration. The excluded subjects were on average older and more often institutionalized. This group probably contained more subjects with physical or mental disabilities or lens opacities that contributed to the poor quality of the transparencies. However, there were only small differences in cardiovascular risk factors between the two groups, suggesting a limited role for selection bias.

The cross-sectional setting prevented inferring causality or chronological order of events. Photographs were not taken synchronized on the cardiac cycle, so there might be variation in vessel diameter due to pulsatility. A variation of 2-17% in vessel diameter has been described.[27] However, because photography was independent of any subject characteristics, this will have caused random misclassification.

Strengths of this study include its population-based design, data collection within a short time-span and the detailed measurement of vessel diameters on $20^0$ stereoscopic fundus transparencies (leading to higher magnification) obtained after pharmacological mydriasis. Both ARIC and Cardiovascular Health Study (CHS) used $45^0$ photographs without pharmacological mydriasis, whereas the Blue Mountains Eye Study used $30^0$ photographs through dilated pupils.[6,15,13] Furthermore, ours seems the first study that used the improved Parr-Hubbard formulas and Littmann's correction to approximate absolute intra-luminal diameters.[20,21]

**Blood pressure and retinal vessel diameters**

When analyzed separately, the impact of blood pressure was more prominent in younger subjects and more in arterioles than venules, as expected. The decrease in arteriolar diameters not only reflects vasoconstriction but also intimal thickening, medial hyperplasia, hyalinization and sclerosis.[28,29] We found smaller decrease in arteriolar diameter (1.1μm and 2.1μm per 10-mmHg increase in systolic and diastolic blood pressure) than reported (1.9μm and 4.3μm).[13] Apart from the abovementioned differences in grading and measuring techniques, this smaller decrease could be explained by the older age distribution of our (55-99 years) versus the ARIC (48-76 years) and the Blue Mountains cohorts (49+ years, only 8% above age 80 years).[6,13] In the older age groups the vessels become progressively rigid and lose their ability to adequately react to blood pressure changes.[30,31]

The association between venular diameter and blood pressure was weak, and stratified on age there was no trend. In our oldest age category the estimate might be unstable due to low numbers.

**Atherosclerosis and retinal vessel diameters**

There was no relationship between the arteriolar diameters and markers of atherosclerosis, except for intima-media thickness. Apart from being a marker of atherosclerosis, intima-media thickness may indicate a response of the artery wall to changes in shear and tensile stress due to hypertension.[32] This is also supported by our data, because after additional adjustment for blood pressure this association became weaker.

We did find a relationship between atherosclerosis and larger venular diameters. A clear patho-physiological explanation for this association is lacking. It is known that in diabetic retinopathy the retinal venules become wider.[33] Other examples of venular dilatation are patients with central retinal vein occlusion, carotid artery narrowing leading to so-called venous stasis retinopathy or deep venous thrombosis in the legs who also have more often atherosclerosis.[34,35] It has been suggested that hyperlipidemia, platelet activation and blood coagulation are involved in the development of both atherosclerosis and venous thrombosis.[35] Whether atherosclerosis induces venular changes, both share common risk factors or occur independently of each other, still needs to be elucidated.

In the CHS study atherosclerosis as measured by ankle-arm index, carotid plaques and intima-media thickness was, contrary to our findings, not related to AVR.[15] Selection bias might have occurred, because they performed carotid ultra-sonography five years prior to fundus photography.[15] In the ARIC study, however, a lower AVR was related to more carotid plaques,[16] and in our study the relationship with AVR persisted for intima-media thickness and carotid plaque-score after adjusting for blood pressures. The relationship between markers of atherosclerosis and AVR is mainly driven by larger venular diameters.

**Inflammation markers, cholesterol levels and retinal vessel diameters**

Higher leukocyte count, higher erythrocyte sedimentation rate, lower HDL levels, higher waist-to-hip ratio and smoking were associated with larger arteriolar and even more strongly with larger venular diameters. Because inflammation also plays an important part in atherosclerosis, vessel widening in these conditions may be due to similar mechanisms. We hypothesize that disruption of the endothelial surface layer (ESL) may underlie the apparent vessel widening. The endothelial surface of all vessels is coated with a matrix of proteoglycans and glycoproteins. This layer (glycocalyx) is directly bound to the plasma-membrane.[36,37] An immobile plasma layer consisting of soluble plasma proteins including glycosaminoglycans is attached to this glycocalyx.

Together these layers are known as the ESL with a thickness ranging from 0.5µm to over 1.0µm.[36] The presence of the ESL may influence several physiological functions of the vessels including flow resistance, barrier function, leukocyte adhesion, coagulation and angiogenesis.[36] Damage to this ESL seems to be the earliest detectable injury to the vascular wall in atherosclerosis.[37] Free radicals as produced by oxidized low-density lipoproteins or activated leukocytes, can disrupt this surface.[37] It could be that the increase in intra-luminal diameter we observed with increasing severity of atherosclerosis or inflammation partly reflects a diminishing ESL.

That higher leukocyte count, lower HDL levels, higher body mass index, higher waist-to-hip ratio and smoking were related to a lower AVR supports the suggestion that the AVR is also a marker of inflammation and endothelial dysfunction.[16]

**Conclusion**

Our data confirmed that elevated blood pressures were associated with smaller arteriolar diameters, but revealed that larger venular diameters were related to atherosclerosis, inflammation and cholesterol levels. Hence, the idea that the AVR overall reflects generalized arteriolar narrowing needs to be re-evaluated by taking into account the separate arteriolar and venular diameters. These data indicate that the venular diameters do not remain constant in different pathological conditions and may play their own independent role in predicting cardiovascular disease. In future research more attention needs to be paid to the role of venules in vascular disease.

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# *Part III*

# *Ophthalmic Epidemiology*

# Chapter 3.1

# Blood Pressure, Atherosclerosis, and the Incidence of Age-related Maculopathy: The Rotterdam Study


## ABSTRACT

***Purpose*** – *To determine whether blood pressure and sub-clinical atherosclerosis are associated with incident age-related maculopathy (ARM).*

***Methods*** – *The study was performed within the Rotterdam Study, a population-based, prospective cohort study in Rotterdam, the Netherlands. A total of 4822 subjects who at baseline were 55 years or older, free of ARM, and who participated in at least one of two follow-up examinations after a mean of 2 and 6½ years, were included in the study. At baseline, blood pressure and the presence of atherosclerosis were determined. ARM was assessed according to the International Classification and Grading System, and defined as large soft drusen with pigmentary changes, or indistinct drusen, or atrophic or neovascular age-related macular degeneration.*

***Results*** – *After a mean follow-up of 5.2 years, 417 subjects were diagnosed with incident ARM. Increased systolic blood pressure or pulse pressure was associated with a higher risk of ARM. Adjusted for age, gender, smoking, total and high-density lipoprotein cholesterol, body mass index, and diabetes mellitus, odds ratios (OR) per 10 mmHg increase were 1.08 (95% confidence interval (CI) 1.03-1.14), and 1.11 (95% CI, 1.04-1.18), respectively. Moreover, different measures of atherosclerosis were associated with the risk of ARM. An increase in carotid wall thickness (OR per SD, 1.15 [95% CI 1.03-1.29]) increased the risk of ARM. Lowest compared to highest tertile of ankle-arm index had an OR of 1.32 (95% CI, 1.00-1.75). A weak association was found between aortic calcifications and the risk of ARM.*

***Conclusions*** – *Elevated systolic blood or pulse pressure, or the presence of atherosclerosis may increase the risk of developing ARM.*

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In a recent report, the National Eye Institute estimated that currently 1.6 million Americans suffer from age-related macular degeneration (AMD), the most common cause of incurable blindness and visual impairment in industrialized countries.[1, 2] Due to ageing of the population, the Institute expects this number to double over the next 30 years. At this moment, treatment options include thermal laser and photodynamic therapy, but they are effective in a minority of patients only.[3, 4] Prevention of AMD is hampered by a lack of knowledge about etiology and modifiable risk factors.[5] Only high-dose supplementation with specific antioxidant nutrients has been shown to slow the development of AMD.[6]

Already in 1937, Verhoeff and Grossman postulated that systemic vascular factors may be involved in the pathogenesis of AMD.[7] More recently, interest in this potential relationship has grown,[8] and a vascular model was proposed in which a process that resembles atherosclerosis causes an accumulation of lipids and subsequently an increase in choroidal vascular resistance.[9] This process would interfere with the high metabolic rate of the retinal pigment epithelium and lead to the development of subretinal deposits (drusen), pigment abnormalities, and finally to the blinding late stages of atrophic or neovascular AMD. Collectively, these early and late fundus signs

are called age-related maculopathy (ARM) according to an international consensus.[10]

Most epidemiological studies have addressed the vascular hypothesis by studying the classical risk factors for cardiovascular disease, like blood pressure, serum cholesterol, and smoking, as well as clinical manifestations of atherosclerosis, such as myocardial infarction. Except for smoking, the results were inconclusive.[11-20] However, very few of these studies were population-based and prospective in design. Only two prevalence studies, including a cross-sectional analysis of the Rotterdam Study, employed direct measurements of atherosclerosis.[21, 22]

To further explore the vascular hypothesis, we studied in a population-based cohort the association of systemic blood pressure and sub-clinical atherosclerosis with the risk of ARM. We used non-invasive techniques for the measurement of atherosclerotic changes and studied prospectively the development of ARM.

## METHODS

### Population

Information on the identification and description of the baseline study population has appeared in previous reports.[23] Briefly, the Rotterdam Study is a population-based prospective cohort study of the frequency and determinants of common cardiovascular, locomotor, neurological, and ophthalmologic diseases.[24] The eligible population (n=10,275) consisted of all inhabitants aged 55 years and older of a suburb of Rotterdam, the Netherlands. Of these, 7983 subjects (78%) agreed to participate in the study. Because the ophthalmologic part of the study became operational after the screening of participants had started, a smaller portion (n=6780) participated in the ophthalmic examination. The study was conducted according to the tenets of the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline interviews and examinations were performed from 1990 to mid 1993, followed by a first follow-up examination from 1993 to 1994. A second follow-up screening took place from mid 1997 to the end of 1999.

### Diagnosis of age-related maculopathy

A detailed description of the diagnostic procedures has been presented elsewhere.[23] Participants underwent a full eye examination, including stereo

35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) centered on field 2 (the fovea) following pharmacological mydriasis. The resulting transparencies were graded with 12.5x magnification according to the International Classification and Grading System for ARM and AMD.[10] In this system, all ARM fundus signs within a standard circle (diameter 6000 μm) around the fovea are recorded. Two graders, trained according to the Wisconsin ARM grading system and having eight years experience, first graded the follow-up transparencies after which these were compared with those taken at baseline. The grading procedures and definitions, as well as the graders, were identical at baseline and at follow-up. Consensus sessions and between-grader comparisons were performed regularly. Weighted kappa values were 0.72 for soft distinct drusen, 0.80 for hyperpigmentation, and 0.58 for hypopigmentation.

ARM was defined as the presence of either large (≥63 μm) soft distinct drusen with pigmentary irregularities, or indistinct (≥125 μm) or reticular drusen, or atrophic or neovascular AMD. Atrophic AMD was defined as any sharply demarcated round or oval area of apparent absence of the RPE, larger than 175 μm, irrespective of distance from the foveola but within the grid, with visible choroidal vessels and no neovascular AMD. Neovascular AMD was defined as the presence of a serous or hemorrhagic neuroretina or RPE detachment and/or a subretinal neovascular membrane and/or a subretinal hemorrhage, and/or a periretinal fibrous scar. Lesions that were considered to be the result of generalized disease, such as diabetic retinopathy, chorioretinitis, high myopia, trauma, congenital diseases, or photocoagulation for reasons other than for neovascular AMD, were excluded from ARM classification.

**Exposure measurement**

Information on smoking habits and current use of medication was derived from the baseline interview. Smoking was categorized as never, former or current. At the research center, height and weight were determined and non-fasting blood samples were drawn. Serum total cholesterol and HDL-cholesterol levels were measured by an automated enzymatic procedure. Diabetes mellitus was considered to be present when subjects currently used oral blood-glucose lowering medication or insulin, or had a non-fasting or post-load glucose level above 11.0 mmol/l.

Blood pressure was measured with a random zero sphygmomanometer at the right brachial artery with the subject in sitting position, and two consecutive measurements were averaged. Pulse pressure was calculated

by taking the difference between systolic and diastolic blood pressure. The systolic blood pressure level of the posterior tibial artery was measured at both sides using an 8-MHz continuous wave Doppler probe (Huntleigh 500 D) and a random-zero sphygmomanometer. The ankle-arm index was calculated by taking the ratio of the systolic blood pressure at the ankle to the systolic pressure at the arm. This was calculated for each leg, and the lowest index was used in the analyses. An ankle-arm index less than 0.90 was considered to indicate peripheral arterial disease.

Wall thickness of the carotid artery was assessed by ultrasonography using a 7.5-MHz linear-array transducer (ATL Ultra-Mark IV), in accordance with the Rotterdam Study ultrasound protocol.[25] Briefly, the intima-media thickness was measured on a longitudinal, two-dimensional ultrasound image of the common carotid artery, the carotid bifurcation, and the internal carotid artery at both the left and right side. When an optimal image of the interface of the anterior (near) and posterior (far) walls was obtained, it was frozen on the R-wave of the ECG, stored on videotape, and digitized using additional dedicated software. Next, the interfaces of the common carotid artery, the carotid bifurcation and the internal carotid artery were marked across a length of 10 mm. The computer calculated the mean and maximum intima-media thickness for both near and far wall. For the analyses, the wall thickness was determined as the mean of the maximum intima-media thickness of near- and far-wall measurements of both the left and right side arteries. The values of each of the three arterial segments were combined after standardization. The ultrasonographers and readers of the images were masked for the case status of the subject.

The common carotid artery, bifurcation, and internal carotid artery were also examined for the presence of atherosclerotic plaques. Plaques were defined as focal widenings of the vessel wall relative to adjacent segments, composed of calcified or non-calcified components. The plaque score reflected the total number of locations where plaques were found and ranged from 0 to 6 (left and right-sided common carotid artery, bifurcation, and internal carotid artery).

Aortic atherosclerosis was diagnosed by detecting calcified deposits in the abdominal aorta on lateral radiographic films of the lumbar spine, as described previously.[26] Calcification was considered present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). The extent of calcification was classified according to the length of the involved area (0, 0.5 to <1, 1 to <2.5, 2.5 to <5, 5 to <10, and ≥10 cm). We considered the first class as reference, the second and third class as mild to

moderate, and the fourth and fifth class as severe calcification.

Finally, a composite score of atherosclerosis was constructed using both the continuous (ankle-arm index and carotid wall thickness) and categorical measurements (number of carotid plaques and aortic calcifications). In order to do so, we transformed all variables to a 10-point scale. For the continuous variables, deciles were created and subjects would get one point per decile. For the categorical variables, we determined what percentage of the study population was in a less severe category and this percentage was converted to points. For example, a person with an ankle-arm index of 1.1 ($6^{th}$ decile), a carotid wall thickness of 0.8 mm ($6^{th}$ decile), carotid plaques at 2 locations (57% of the population had less than 2 locations), and 1 to < 2.5 cm of aortic calcifications (43% had less than this), has a score of 6 + 6 + 5.7 + 4.3 = 22.

**Study sample**

Of the 6780 participants in the ophthalmic part of the baseline study, 6477 (95.5%) persons underwent fundus photography and 6418 (94.7%) persons had gradable fundus transparencies in at least one eye. Prevalent ARM was diagnosed in 582 (9.1%) subjects, including 106 cases of AMD. This resulted in a cohort of 5836 subjects at risk who were free of ARM, i.e. subjects with no drusen, only hard or distinct drusen, or pigmentary abnormalities only. Of this cohort, 283 (4.8%) subjects died before the first follow-up examination and another 789 (13.5%) subjects died before the second follow-up. Of those alive at the first screening (n=5553), 46 subjects were lost to follow-up, 905 refused to participate, and 13 had ungradable photographs. Of those alive at the second follow-up (n=4764), 15 subjects were lost to follow-up, 1267 refused to participate, and 47 had ungradable photographs. In total, 4822 subjects (83% of those at risk) participated in at least one follow-up exam. Of these, blood pressure measurements were missing in 50 participants, ankle-arm index in 394 participants, carotid wall thickness in 770 participants, plaques in carotid artery in 1572 participants, and aortic calcifications in 497 participants. The main cause of missing data on ultrasonography was restricted availability of technicians, which was irrespective of a subject's exposure and disease status.

Incidence of ARM was defined as absence of ARM in either eye at baseline and presence of ARM in at least one eye at follow-up.

**Data analysis**

Analysis of variance, adjusted for age and gender, was used to compare

baseline characteristics of eligible subjects participating in at least one follow-up examination with those who were alive at the time of examination but did not participate.

We studied the associations of baseline blood pressure and atherosclerosis with incident ARM in subjects with no ARM at baseline. Logistic regression analysis was used with time of follow-up included in every model. Systolic blood pressure, diastolic blood pressure, and pulse pressure were entered in the model either as a continuous or a categorical variable. In the first case, the regression coefficient was expressed per 10 mmHg increase. In the second case, three dummy variables were defined based on absolute values of blood pressure with predefined cut-off points. In order to detect a J-shaped relationship, the second category of diastolic blood pressure (65-74 mmHg) was used as reference. Ankle-arm index was analyzed with the predefined cut-off point of 0.9, as well as in tertiles. We studied wall thickness of the carotid artery both as a continuous variable (per SD) and as a categorical variable (tertiles). Plaques in the carotid artery were analyzed both continuously (in number of plaques) and in categories. Aortic calcifications were studied in categories only and the atherosclerosis composite score was analyzed in quartiles.

Initially, the regression analysis was adjusted for age and gender (model 1). In model 2, additional adjustment was made for smoking (current/former/never), diabetes mellitus (yes/no), total cholesterol and HDL-cholesterol (per mmol/l), and body mass index (per kg/m$^2$). In model 3, we also adjusted for the composite score of atherosclerosis in the blood pressure analyses, and for systolic and diastolic blood pressure in the atherosclerosis analyses. The associations are presented as odds ratios (OR), which can be interpreted as relative risks, with 95% confidence intervals (CI). All analyses were performed using SPSS statistical software version 11 (SPSS Inc., Chicago, Illinois).

## RESULTS

The baseline characteristics of the eligible study cohort, adjusted for age and gender, are presented in Table 1. Of the eligible subjects that were alive at the time of follow-up examination, 1014 (17.4%) did not participate. Compared with participants, these subjects were significantly older, included more current smokers, had more often diabetes mellitus, used more antihypertensive medication, and had more severe atherosclerosis.

The average time between baseline and first follow-up examination was 2.0 years, and between baseline and the second one 6.5 years. Follow-

up of all participants was on average 5.2 years, with a range of 1.0 to 9.7 years (median 6.3 years). During this period, 419 subjects were diagnosed with incident ARM, of whom the majority had early ARM and 14 had AMD (four atrophic and 10 neovascular AMD). Incident cases with early ARM had either large soft drusen with pigmentary irregularities (n=261), or indistinct drusen without (n=109), or with pigmentary irregularities (n=35). One of the incident AMD cases developed AMD at the first follow-up examination and 13 at second. Six cases had soft distinct drusen or pigmentary abnormalities at baseline, while eight showed early ARM at the first follow-up examination. The incidence of ARM did not differ between the study sample and participants with missing data on atherosclerosis (*P*=0.17, adjusted for age and gender).

In Table 2, the odds ratios (OR) of incident ARM associated with

**Table 1**. Baseline characteristics of subjects at risk for age-related maculopathy. Values are age-adjusted means or percentages ± standard error

| | | Participants (n=4822) | | | Non-participants† (n=1014) | | |
|---|---|---|---|---|---|---|---|
| Age, years | | 67.1 | ± | 0.1 | 73.9 | ± | 0.3** |
| Female, % | | 59.1 | ± | 0.01 | 59.0 | ± | 0.02 |
| Body mass index, kg/m$^2$ | | 26.4 | ± | 0.1 | 26.1 | ± | 0.1* |
| Smoking, % | Never | 35.2 | ± | 0.01 | 30.5 | ± | 0.01** |
| | Former | 43.3 | ± | 0.01 | 38.6 | ± | 0.02** |
| | Current | 21.6 | ± | 0.01 | 30.9 | ± | 0.01** |
| Diabetes mellitus, % | | 9.8 | ± | 0.4 | 13.5 | ± | 1.0** |
| Mean total cholesterol, mmol/l | | 6.67 | ± | 0.02 | 6.56 | ± | 0.04* |
| Mean HDL cholesterol, mmol/l | | 1.35 | ± | 0.01 | 1.34 | ± | 0.01 |
| Systolic blood pressure, mmHg | | 138.6 | ± | 0.3 | 140.4 | ± | 0.7* |
| Diastolic blood pressure, mmHg | | 73.8 | ± | 0.2 | 74.1 | ± | 0.4 |
| Anti hypertensive medication, % | | 30.9 | ± | 0.01 | 34.7 | ± | 0.02* |
| Ankle-arm index | | 1.08 | ± | 0.003 | 1.02 | ± | 0.007** |
| Wall thickness common carotid artery, mm | | 0.79 | ± | 0.002 | 0.81 | ± | 0.005** |
| No. of carotid plaques, % | 0 | 40.9 | ± | 0.01 | 40.1 | ± | 0.02 |
| | 1-3 | 44.7 | ± | 0.01 | 38.7 | ± | 0.02** |
| | 4-6 | 14.4 | ± | 0.01 | 21.2 | ± | 0.02** |
| Atherosclerosis composite score | | 26.2 | ± | 0.02 | 28.4 | ± | 0.04** |

† Not participating, but alive at the moment of screening
* P-value < 0.05
** P-value < 0.01

**Table 2**. Adjusted odds ratios of incident age-related maculopathy associated with baseline blood pressure (BP) levels.

| | | Number of | | Adjusted Odds Ratio (95% Confidence Interval) | | |
|---|---|---|---|---|---|---|
| | | Subjects | Cases | Model 1* | Model 2† | Model 3‡ |
| Systolic BP, per 10 mm Hg increase | | 4772 | 416 | 1.06 (1.01, 1.12) | 1.08 (1.03, 1.14) | 1.07 (1.01, 1.13) |
| Systolic BP, categories (mm Hg) | < 120 | 977 | 52 | 1.00 | 1.00 | 1.00 |
| | 120 - 139 | 1689 | 143 | 1.47 (1.06, 2.05) | 1.61 (1.14, 2.27) | 1.63 (1.12, 2.37) |
| | 140 - 159 | 1366 | 140 | 1.70 (1.22, 2.39) | 1.86 (1.31, 2.66) | 1.81 (1.23, 2.67) |
| | ≥ 160 | 740 | 81 | 1.85 (1.27, 2.69) | 2.07 (1.40, 3.07) | 2.08 (1.36, 3.20) |
| Diastolic BP, per 10 mm Hg increase | | 4772 | 416 | 1.05 (0.96, 1.15) | 1.07 (0.97, 1.17) | 1.07 (0.97, 1.18) |
| Diastolic BP, categories (mm Hg) | < 65 | 940 | 86 | 1.05 (0.79, 1.39) | 1.03 (0.77, 1.38) | 0.92 (0.67, 1.27) |
| | 65 - 74 | 1675 | 144 | 1.00 | 1.00 | 1.00 |
| | 75 - 84 | 1367 | 114 | 1.02 (0.79, 1.32) | 1.04 (0.80, 1.36) | 0.97 (0.73, 1.29) |
| | ≥ 85 | 790 | 72 | 1.23 (0.91, 1.67) | 1.27 (0.93, 1.73) | 1.23 (0.88, 1.71) |
| Pulse pressure, per 10 mm Hg increase | | 4772 | 416 | 1.09 (1.02, 1.15) | 1.11 (1.04, 1.18) | 1.08 (1.00, 1.16) |
| Pulse pressure, categories (mm Hg) | < 50 | 1013 | 54 | 1.00 | 1.00 | 1.00 |
| | 50 - 64 | 1610 | 140 | 1.45 (1.04, 2.02) | 1.50 (1.07, 2.11) | 1.26 (0.88, 1.81) |
| | 65 - 79 | 1309 | 129 | 1.51 (1.07, 2.12) | 1.54 (1.08, 2.19) | 1.41 (0.97, 2.06) |
| | ≥ 80 | 840 | 93 | 1.59 (1.09, 2.30) | 1.73 (1.18, 2.54) | 1.41 (0.92, 2.15) |

* Adjusted for age and gender

† Additional adjustment for smoking, total and HDL cholesterol, body mass index, and diabetes mellitus

‡ Additional adjustment for a composite score of atherosclerosis

**Table 3**. Adjusted odds ratios of age-related maculopathy associated with ankle-arm index.

| | | Number of | | Adjusted Odds Ratio (95% Confidence Interval) | | |
|---|---|---|---|---|---|---|
| | | Subjects | Cases | Model 1* | Model 2† | Model 3‡ |
| Ankle-arm index, categories | ≥ 0.9 | 3833 | 326 | 1.00 | 1.00 | 1.00 |
| | < 0.9 | 595 | 54 | 0.90 (0.66, 1.24) | 0.90 (0.64, 1.25) | 0.81 (0.52, 1.27) |
| Ankle-arm index, tertiles | 1st (highest) | 1604 | 118 | 1.00 | 1.00 | 1.00 |
| | 2nd | 1560 | 133 | 1.17 (0.90, 1.52) | 1.17 (0.89, 1.54) | 1.10 (0.83, 1.45) |
| | 3rd (lowest) | 1264 | 129 | 1.32 (1.00, 1.75) | 1.39 (1.04, 1.84) | 1.26 (0.94, 1.69) |

* Adjusted for age and gender

† Additional adjustment for smoking, total and HDL cholesterol, body mass index, and diabetes mellitus

‡ Additional adjustment for systolic and diastolic blood pressure

baseline blood pressure are shown. When adjusted for age and gender, elevated systolic blood pressure was associated with an increased risk of ARM (OR per 10 mmHg increase: 1.06, 95% CI 1.01, 1.12). When additional adjustments were made for smoking, total and HDL cholesterol, body mass index, diabetes mellitus, and the composite score of atherosclerosis, the association remained statistically significant. Diastolic blood pressure was also associated with ARM, but this did not reach statistical significance (OR per 10 mmHg increase: 1.05, 95% CI 0.96, 1.15, adjusted for age and gender). Additional adjustment did not change this relationship. Also, taking the lowest category as reference instead of the second did not change the risk estimates. Pulse pressure was positively associated with the risk of ARM, both as a continuous and as a categorical variable. Adjustment for the composite score of atherosclerosis, however, attenuated the association with categories of pulse pressure to non-significant levels. Excluding subjects who used blood pressure-lowering medication at baseline did not substantially alter the results (data not shown).

Table 3 presents the association between ankle-arm index and risk of ARM. Peripheral atherosclerosis, defined as an ankle-arm index lower than 0.9, was not associated with ARM. However, when analyzing the ankle-arm index in tertiles, the lowest compared with the highest tertile showed a borderline significant increased risk of ARM (OR 1.32, 95% CI 1.00, 1.75). The association was a little stronger when additional adjustments were made, but became non-significant when adjusted for systolic and diastolic blood pressure.

Table 4 shows the relationship between measures of atherosclerosis in

the carotid artery and risk of ARM. Increased wall thickness of the common carotid artery, both as a continuous (per SD) and as a categorical variable, significantly increased the risk of ARM. Per SD (0.15 mm) of wall thickness, the OR was 1.15 (95% CI 1.03, 1.28; adjusted for age and gender). The highest tertile of carotid wall thickness compared with the lowest tertile had an OR of 1.45 (95% CI 1.07, 1.97). Additional adjustment for cardiovascular risk factors (model 2), including systolic and diastolic blood pressure (model 3) did not substantially alter the results. Plaques in the carotid artery were also associated with an increased risk of ARM. Compared with no plaques, four to six plaques in the right and left carotid artery increased the risk of ARM with nearly 50% (OR 1.46, 95% CI 1.02, 2.09, adjusted for age and gender). Additional adjustment for systolic and diastolic blood pressure decreased this risk estimate to a slight extent.

The relation between calcification of the abdominal aorta and ARM is shown in Table 5. A positive but statistically not significant association was found between aortic calcification and the incidence of ARM. Adjusted for all potential confounders, severe calcifications compared to none showed an OR of 1.39 (95% CI 0.98, 1.98).

Finally, in Table 6, the analysis of the composite score of atherosclerosis is presented. The highest score compared with the lowest carried an OR

**Table 4**. Adjusted odds ratios of age-related maculopathy associated with carotid artery wall thickness and presence of plaques.

| | | Number of | | Adjusted Odds Ratio (95% Confidence Interval) | | |
|---|---|---|---|---|---|---|
| | | Subjects | Cases | Model 1* | Model 2† | Model 3‡ |
| Wall thickness carotid artery, per SD | | 4052 | 357 | 1.15 (1.03, 1.28) | 1.15 (1.03, 1.29) | 1.11 (0.99, 1.25) |
| Wall thickness carotid artery, tertiles | 1st | 1489 | 94 | 1.00 | 1.00 | 1.00 |
| | 2nd | 1381 | 127 | 1.26 (0.95, 1.68) | 1.33 (0.99, 1.79) | 1.30 (0.96, 1.75) |
| | 3rd | 1182 | 136 | 1.45 (1.07, 1.97) | 1.53 (1.12, 2.11) | 1.42 (1.03, 1.97) |
| Plaques in carotid artery, per plaque | | 3250 | 290 | 1.06 (0.98, 1.14) | 1.06 (0.98, 1.15) | 1.04 (0.96, 1.13) |
| Plaques in carotid artery, categories | 0 | 1377 | 107 | 1.00 | 1.00 | 1.00 |
| | 1-3 | 1429 | 127 | 1.02 (0.77, 1.34) | 1.02 (0.77, 1.35) | 0.97 (0.72, 1.29) |
| | 4-6 | 444 | 56 | 1.46 (1.02, 2.09) | 1.49 (1.03, 2.17) | 1.36 (0.93, 1.99) |

* Adjusted for age and gender

† Additional adjustment for smoking, total and HDL cholesterol, body mass index, and diabetes mellitus

‡ Additional adjustment for systolic and diastolic blood pressure

**Table 5**. Adjusted odds ratios of age-related maculopathy associated with aortic calcifications.

| | | Number of | | Adjusted Odds Ratio (95% Confidence Interval) | | |
|---|---|---|---|---|---|---|
| | | Subjects | Cases | Model 1* | Model 2† | Model 3‡ |
| Aortic calcification, categories | No | 1556 | 85 | 1.00 | 1.00 | 1.00 |
| | Mild-Moderate | 1574 | 127 | 1.24 (0.93, 1.66) | 1.24 (0.92, 1.68) | 1.36 (0.98, 1.88) |
| | Severe | 1195 | 108 | 1.23 (0.90, 1.68) | 1.28 (0.92, 1.76) | 1.39 (0.98, 1.98) |

* Adjusted for age and gender
† Additional adjustment for smoking, total and HDL cholesterol, body mass index, and diabetes mellitus
‡ Additional adjustment for systolic and diastolic blood pressure

of 1.53 (95% CI 1.08, 2.18). Additional adjustment for cardiovascular risk factors only marginally reduced this risk estimate, but adjustment for blood pressure made it non-significant (OR 1.41, 95% CI 0.95, 2.09).

## DISCUSSION

In this prospective cohort study, we observed that high systolic blood pressure or high pulse pressure, as well as the presence of atherosclerosis were associated with an increased risk of ARM. The association was overall not altered when adjusted for confounders and was strongest for atherosclerosis in the carotid artery.

In this study, we used the combination of early and late signs of ARM as incident outcome. Since subjects with early stages at baseline were excluded, very few (n=14) developed AMD within the five-year follow-up period and the large majority (97%) of incident cases had early ARM. When participants with incident AMD were excluded from the analysis, the same results were obtained (data not shown). We may therefore conclude that blood pressure and atherosclerosis promote the development of drusen and other signs of early ARM, and not (only) the progression of early ARM to neovascular AMD, as was suggested by some authors.[27] A separate analysis with only neovascular AMD as outcome was not possible due to low numbers. Given the well-documented risk of early ARM to progress to the blinding stage of AMD, it seems justified to assume that risk factors of early ARM also increase the risk of AMD.[28, 29]

A concern in this as well as other follow-up studies is selective non-response. Because the international standard for the diagnosis of ARM in epidemiological studies relies on fundus photography,[10] follow-up depends

on a subject's participation in the eye examination. According to the analysis of baseline characteristics, subjects who did not participate in the follow-up exams had more cardiovascular risk factors and more atherosclerosis. Therefore, subjects with severe atherosclerosis were underrepresented in the studied sample. This reduction in the range of atherosclerosis severity has made it more difficult to find an association.

Sixteen percent of participants had missing data on carotid wall thickness and plaques, which was mainly due to logistic problems and to technical difficulties in visualization of the carotid artery. Since these reasons were not related to carotid wall thickness, we do not think that this has biased our results. Still, it is possible that some error has occurred in the measurement of atherosclerosis. Such a measurement error would have led to an underestimation of the true relationship with ARM, provided that the error occurred to the same extent among subjects with ARM and those without. Another question to be discussed is whether ankle-arm index and ultrasonographic measurement of carotid wall thickness are true indicators of atherosclerosis. The relation between ankle-arm index and atherosclerosis seems well established.[30] Increase in the carotid intima-media thickness may also reflect hypertrophy of the vessel wall as a response to hypertensive stress. Since many studies have shown that increased carotid wall thickness is independently of hypertension associated with both cardiovascular risk factors and cardiovascular events, it can be regarded as a valid indicator of atherosclerosis.[25, 31]

An association between blood pressure or hypertension and prevalent AMD was reported earlier by three case-control studies, the Eye Disease

**Table 6**. Adjusted odds ratios of age-related maculopathy associated with a composite score of atherosclerosis.

| | | Number of | | Adjusted Odds Ratio (95% Confidence Interval) | | |
|---|---|---|---|---|---|---|
| | | Subjects | Cases | Model 1* | Model 2† | Model 3‡ |
| Composite score of atherosclerosis, quartiles | 1st | 975 | 60 | 1.00 | 1.00 | 1.00 |
| | 2nd | 1092 | 87 | 1.16 (0.82, 1.65) | 1.24 (0.87, 1.78) | 1.17 (0.82, 1.69) |
| | 3rd | 1041 | 96 | 1.20 (0.85, 1.70) | 1.27 (0.88, 1.84) | 1.14 (0.78, 1.67) |
| | 4th | 1040 | 127 | 1.53 (1.08, 2.18) | 1.63 (1.12, 2.37) | 1.41 (0.95, 2.09) |

* Adjusted for age and gender

† Additional adjustment for smoking, total and HDL cholesterol, body mass index, and diabetes mellitus

‡ Additional adjustment for systolic and diastolic blood pressure

Case-Control Study,[12] the AMD Risk Factors Study,[15] and the Age-Related Eye Disease Study.[14] Also, the Framingham Eye Study found an association between prevalent ARM and hypertension diagnosed 25 years before.[11] On the other hand, no association between blood pressure and prevalence of ARM was found in several population-based studies.[16-21] The only prospective, population-based study on this association so far, the Beaver Dam Eye Study, found that both systolic blood pressure and hypertension were significantly related to the incidence of retinal pigment epithelial depigmentation, but not of drusen.[13] Loss of power might be the explanation, because the number of incident cases with early ARM in their cohort was about half that in our study.

The association between ultrasonographically determined atherosclerosis and the incidence of ARM has not yet been studied, as far as we know. Klein et al. have studied the prevalence of ARM in the population-based ARIC study, in which data on carotid intima-media wall thickness and carotid plaques were available.[21] They did find a statistically significant association between carotid plaques and retinal depigmentation, but, referring to the large number of associations studied, they concluded that atherosclerosis was overall unrelated to ARM. Also, they used non-stereoscopic 45º fundus photographs taken through a non-pharmacologically dilated pupil of only one eye, which may have resulted in a decreased detection of ARM.[32] In an earlier cross-sectional analysis of data from the Rotterdam Study, we found that subjects with plaques in the carotid bifurcation were 4.5 times more likely to have AMD.[22] Also, an ankle-arm index below 0.9 was significantly (OR 2.0; 95% CI 1.2, 3.2) associated with the presence of AMD. However, in the latter study the early signs of ARM were not included, and the numbers of AMD were low with corresponding wide confidence intervals. Moreover, given the cross-sectional design no causal inferences could be made.

Not all measures of atherosclerosis yielded the same results. The strongest association with ARM was observed for carotid wall thickness and carotid plaques, while no association was found for calcifications in the abdominal aorta and peripheral arterial disease. Considering this difference, one might hypothesize that atherosclerosis of the cerebral circulation is more important for the risk of ARM than atherosclerosis of the aorta or peripheral arteries.

There are several ways in which atherosclerosis may be related to ARM. Carotid atherosclerosis may lead to stenosis and, in the end, to a diminished blood flow to the ophthalmic artery and the choroidal and retinal circulation. Considering the low prevalence of carotid stenosis in our cohort,

this explanation seems not plausible. It is more likely that the atherosclerosis we measured reflects a similar process in the choroidal vessels under the retina. Thickening and stiffening of the vessel wall will result in a decreased lumen diameter, an increased blood flow resistance, and a decreased tissue perfusion. This process may then either directly impair the functioning of the retinal pigment epithelium, which is responsible for the metabolism of rod and cone outer segments, or may lead to leakage and deposition of proteins and lipids due to elevated hydrostatic pressure, as was proposed by Friedman.[9] A decreased choriocapillary density was demonstrated in ageing human eyes, especially in those with ARM.[33] Moreover, in patients with ARM a reduced choroidal perfusion was shown by direct measurement of the choroidal blood flow.[34-37] In summary, multiple lines of evidence suggest a role for atherosclerosis in the pathophysiology of ARM.

The question should be answered whether high blood pressure is a risk factor for ARM in itself, or high blood pressure is a risk factor for ARM only through its association with atherosclerosis. To disentangle this relationship, we put both determinants in the same model. Adjustment for the composite score of atherosclerosis did not change the association between systolic or diastolic blood pressure and ARM. The association with pulse pressure was somewhat attenuated, possibly indicating that pulse pressure has more overlap with atherosclerosis. Additional adjustment for systolic and diastolic blood pressure altered only to a slight degree the risk estimates for the association of atherosclerosis with ARM. The interpretation of these analyses might be that high blood pressure and atherosclerosis independently of each other increase the risk of ARM. However, since both determinants are strongly linked and both were measured at the same time, the previous analyses will not be sufficient to determine the exact order of the pathophysiological pathway.

The level of oxidative defense could disturb the association between atherosclerosis and ARM. Because oxidative stress is implicated both in the etiology of ARM[38] and in the pathogenesis of atherosclerosis,[39] antioxidants may act as a confounder in the observed association. However, this confounder is less likely to explain the relationship between blood pressure and ARM, which was independent of atherosclerosis.

In conclusion, in this large prospective cohort study we showed that high systolic blood pressure, high pulse pressure, or the presence of subclinical atherosclerosis increases the risk of ARM. The magnitude of the risk estimates varied, with a maximal OR of 2.1. Because of the high prevalence of hypertension and atherosclerosis in the population, the impact of these

factors on the total incidence of ARM may still be large. Our results suggest that a reduction in the occurrence of hypertension and atherosclerosis may add to the prevention of this blinding disease.

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# Chapter 3.2

# Retinal Vessel Diameters and the Risk of Incident Age-related Macular Disease: The Rotterdam Study


## ABSTRACT

***Purpose*** *– To study the relationship between retinal vessel diameters and incident age-related macular disease (iAMD)*

***Design*** *– prospective population-based cohort study*

***Participants*** *– Persons (55 years and older) from the Rotterdam Study, who participated at the baseline (1990-1993) and one of two follow-up examinations (1993-1994 and 1997-1999).*

***Methods*** *– In the current analysis, 4345 participants were included, who were free of AMD at baseline and had gradable macular transparencies both at baseline and follow-up examination. Also, on digitized baseline images arteriolar and venular diameters were measured. Stereoscopic transparencies of the macular region were graded according to the International Classification and Grading System. Incidence of AMD was defined as the development of soft distinct drusen with pigmentary changes, or indistinct or reticular drusen with or without pigmentary changes (early iAMD), or atrophic or neovascular AMD (late iAMD). Logistic regression models were used to assess these associations adjusting for age, gender, follow-up time and additionally for smoking, body mass index, intima-media thickness, systolic blood pressure, total and HDL cholesterol.*

***Main Outcome Measures*** *– Incidence of AMD*

***Results*** *– After a mean follow-up time of 5.2 years, a total of 374 persons developed early and late iAMD. Neither arteriolar nor venular diameters were related to the risk of iAMD. The odds ratio (OR) per standard deviation (SD) decrease in arteriolar diameter was 1.03 (95% confidence interval (CI): 0.93-1.15) and per SD increase in venular diameter was 1.04 (95% CI: 0.93-1.16). After categorizing the retinal vessel diameters into quintiles there was no trend. After stratifying on age, only subjects 75 years or older with smaller arteriolar diameters were at an increased risk of iAMD: OR per SD decrease in arteriolar diameter adjusted for age, gender and follow-up time: 1.30 (95% CI: 1.01-1.67).*

***Conclusion*** *– Overall retinal vessel diameters were not related to the risk of iAMD in this general elderly population.*


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Since the introduction of the vascular hypothesis by Verhoeff and Grossman, the exact contribution of vascular factors to the development of age-related macular disease (AMD) still remains unclear.[1, 2] Most epidemiological studies have addressed this hypothesis by studying classical cardiovascular risk factors such as blood pressure and markers of atherosclerosis and resulting data were inconsistent.[3-10] This might be partly due to the fact that most non-invasive markers of vascular pathology are related to larger vessels outside the eye and may not represent local vascular abnormalities.

The role of local vascular factors in the pathogenesis of AMD is plausible given the choroidal thinning and increased resistance of the choroidal vessels caused by an increase in scleral rigidity in aging.[11-15] The choroidal circulation, that primarily nurtures the outer retina and its pigment epithelium, has been explored using laser Doppler flow measurements in cross-sectional clinic-based studies on a small number of persons with and without AMD.[12-14, 16, 17] It still remains to be elucidated whether hampered choroidal blood flow is causative or reflects a change secondary to the loss of cellular components that occurs with aging and is accentuated in AMD.[14]

Our present paradigm is that hypertensive damage, apart from affecting the choroidal vessels, is seen in the retinal vessel diameters.[2, 18] Recently

a semi-automated system was developed to measure retinal vessel diameters accurately and objectively.[18] Both the Blue Mountains Eye Study and the Beaver Dam Eye Study used this method and found no association between retinal arteriolar diameters and incident AMD (iAMD; odds ratio 1st versus 5th quintile of arteriolar diameters: 1.4 (0.3-5.7) and 1.8 (0.6-5.4), respectively).[19, 20] They did not use the improved Parr-Hubbard formulas[21] nor corrected for differences in magnification[22] due to the optical system of the eye to approximate absolute diameters. Using the improved formulas for this semi-automated system we have recently shown that smaller arterioles are more related to higher blood pressure, whereas larger venules to atherosclerosis and inflammation.[22] So we tested the hypothesis that smaller arteriolar or larger venular diameters are associated with an increased risk of iAMD.

## METHODS AND MATERIALS

### Population

This investigation was part of the Rotterdam Study, a population-based cohort study (n = 10,275) with a response rate of 78%.[23] Because the ophthalmic part became operational after the screening of participants had started, a smaller proportion (n = 6780) participated in the ophthalmic examination. After the baseline phase (1990-1993) the first follow-up examination was conducted between 1993-1994 and the second one between 1997-1999. The study was conducted according to the tenets of the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants.

### Definition of age-related macular disease

Grading and definitions of AMD have been described extensively in previous reports.[24] Briefly, stereoscopic macular transparencies centered on the fovea (stereo 35° fundus photography; Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan), obtained after pharmacological mydriasis, were used to diagnose AMD. The resulting transparencies were graded with 12.5x magnification according to the International Classification and Grading System for AMD.[25] In this system, all AMD fundus signs within a standard circle (diameter 6000 μm) around the fovea are recorded. Two graders, trained according to the Wisconsin AMD grading system and each having eight years experience, first graded the follow-up transparencies after which these were compared with those taken at baseline. The grading pro-

cedures and definitions, as well as the graders, were identical at baseline and at follow-up. Early AMD was defined as the presence of either soft distinct drusen with pigmentary irregularities, or indistinct or reticular drusen with or without pigmentary irregularities, excluding the end-stages; late AMD as an atrophic or neovascular end-stage in either eye of the participant.24 Incidence of AMD was defined as absence of any AMD in either eye at baseline and presence of AMD in at least one eye at follow-up.

**Retinal vessel measurements**

At baseline, also fundus transparencies (pharmacological mydriasis, 200 field, Topcon TRC-SS2, Topcon Optical Company, Tokyo, Japan) centered on the optic disc were obtained. After digitizing these, per person the image with the best quality was analyzed with a semi-automated system (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison) by four trained graders masked for the endpoints.[22] On one eye of each participant both the arteriolar and venular blood column diameters were measured in μm.[18] We used the improved Parr-Hubbard formula to compute their sum values and additionally adjusted for refractive errors using Littmann's formula.[21, 26] For comparison with previous publications we also calculated the arteriolar-to-venular ratio (AVR) as the ratio of the sum arteriolar to the sum venular diameters.

**Other variables**

The average of two blood pressure measurements in sitting position at the right brachial artery with a random-zero sphygmomanometer was taken. Information on smoking habits was derived from the baseline interview. Smoking was categorized as never, former or current. Body mass index was computed as weight divided by height squared (kg/m2). Intima-media thickness (in mm) was measured in the common carotid arteries using ultra-sonography.[27] Non-fasting serum total cholesterol and high-density lipoprotein (HDL) concentrations were determined by an automated enzymatic procedure.[28]

**Statistical methods**

Participants with gradable transparencies were compared to those with ungradable ones by analysis of covariance (ANCOVA), adjusted for age and gender. Associations between baseline retinal arteriolar and venular diameters as well as AVR and iAMD were examined with logistic regression models, adjusted for age, gender and follow-up time, and additionally for

other cardiovascular risk factors. We studied retinal vessel diameters both as categorized (in quintiles) and continuous (per standard deviation (SD)) variable. Follow-up time was calculated using the dates on which the baseline and follow-up photographs were taken. Stratification on age was performed, because the retinal vessels become increasingly rigid and sclerotic at older age.[22] All analyses were performed using SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

Of the 6780 participants that participated in the ophthalmic part of the baseline Rotterdam Study, 6418 had gradable macular transparencies at baseline. After excluding persons with prevalent AMD (n = 582; including 106 late AMD) and those who had no or ungradable macular transparencies at the follow-up examinations, 4822 participants were free of AMD in both eyes at baseline, of whom 477 participants had no or ungradable transparencies for retinal vessel measurements. Thus we studied 4345 participants free of AMD at baseline in either eye with transparencies for both iAMD grading

**Table 1**. Baseline characteristics. Age and gender adjusted means (± standard error) or percentages

| | **Participants* with both gradable macular and disc transparencies** | **Participants* with gradable macular but no or ungradable disc transparencies** | **Non-participants†** |
|---|---|---|---|
| N (subjects) | 4345 | 477 | 1958 |
| Age (years) | 67 (0.13) | 72‡ (0.38) | 75‡ (0.19) |
| Gender (% female) | 60 | 59 | 59 |
| Institutionalized (%) | 5 | 8‡ | 12‡ |
| Diabetes Mellitus (%) | 9.8 | 11.2 | 11.8‡ |
| Smoking (% current) | 20.8 | 21.1 | 28.2‡ |
| BMI§ (kg/m²) | 26.4 (0.06) | 26.2 (0.17) | 26.1‡ (0.09) |
| Systolic blood pressure (mm Hg) | 139.0 (0.32) | 139.8 (0.99) | 140.0 (0.68) |
| Intima-media thickness (mm) | 0.80 (0.003) | 0.80 (0.007) | 0.81‡ (0.004) |
| Total cholesterol (mmol/l) | 6.64 (0.02) | 6.71 (0.06) | 6.52‡ (0.03) |
| HDL\|\| cholesterol (mmol/l) | 1.35 (0.01) | 1.37 (0.02) | 1.35 (0.01) |

* Participated both at baseline and follow-up examinations
† Participated at baseline but none of the both follow-up examinations
‡ Statistically significant compared to the first column at $p < 0.05$.
§ BMI = body mass index
|| HDL = high-density lipoprotein

and baseline retinal vessel measurements. Table 1 shows the age and gender adjusted baseline characteristics of the study population.

During 24,540 person-years of follow-up, 363 participants developed early iAMD and 11 late iAMD. The average time between baseline and first follow-up examination was 2.0 years, and between baseline and the second one 6.5. Follow-up of all participants was on average 5.2 years, with a range of 1.0 to 9.7 years. The mean arteriolar diameter at baseline was 149.6μm (SD: 14.4), the venular 226.0μm (SD: 20.6) and the AVR 0.66 (SD: 0.06).

Table 2 shows that neither retinal arteriolar nor venular diameters were related to the risk of iAMD, nor was the resulting AVR. Excluding persons with late iAMD did not alter the results substantially. After additional adjustments for cardiovascular risk factors the ORs of iAMD per SD decrease in arteriolar diameters were 0.95 (95% CI: 0.84-1.08), per SD increase in venular diameters 1.08 (95% CI: 0.95-1.22) and per SD decrease in AVR 1.02 (95% CI: 0.91-1.16). Table 3 also presents these analyses after categorizing retinal vessel diameters into quintiles. Comparing the lowest to the highest quintile of arteriolar diameters resulted in an OR of 1.13. For the venular diameters the highest compared to lowest quintile gave an OR of 1.09. Quintiles of AVR did show a trend, which disappeared after additional adjustments for blood pressure, atherosclerosis and smoking. Retinal vessel diameters were neither related to the progression of early to late AMD, nor to the incidence of specific AMD lesions (data not shown).

Smaller arteriolar diameters increased the risk of iAMD among participants 75 years and older (table 4). However, after additional adjustments for cardiovascular risk factors the OR of iAMD per SD decrease in arteriolar diameter was 1.24 (95% CI: 0.94-1.63).

**Table 2**. Odds ratios (95% confidence interval) of incident age-related macular disease (AMD) adjusted for age, gender and follow-up time per standard deviation (SD) difference in retinal vessel diameters or arteriolar-to-venular ratio

| | **All AMD** | **Early AMD** |
|---|---|---|
| N (cases) | 374 | 363 |
| Arteriolar narrowing* | 1.03 (0.93-1.15) | 1.08 (0.96-1.20) |
| Venular dilatation† | 1.04 (0.93-1.16) | 1.02 (0.91-1.14) |
| Arteriolar-to-venular ratio‡ | 1.08 (0.96-1.20) | 1.11 (0.99-1.23) |

* Per SD decrease in arteriolar diameters

† Per SD increase in venular diameters

‡ Per SD decrease in arteriolar-to-venular ratio

**Table 3**. Odds ratios (95% confidence interval) for incident age-related macular disease (AMD) in quintiles of retinal vessel diameters or arteriolar-to-venular ratio

| Quintiles | Model I* | Model II† |
|---|---|---|
| Arteriolar diameter | | |
| 5 (largest) | 1.00 | 1.00 |
| 4 | 0.83 (0.58-1.18) | 0.77 (0.53-1.13) |
| 3 | 0.98 (0.69-1.38) | 0.85 (0.58-1.23) |
| 2 | 0.98 (0.69-1.38) | 0.92(0.63-1.33) |
| 1 (smallest) | 1.13 (0.81-1.57) | 0.91 (0.63-1.32) |
| Venular diameter | | |
| 1 (smallest) | 1.00 | 1.00 |
| 2 | 1.04 (0.74-1.46) | 0.95 (0.65-1.39) |
| 3 | 1.08 (0.77-1.52) | 1.10 (0.76-1.61) |
| 4 | 1.10 (0.78-1.55) | 1.15 (0.79-1.68) |
| 5 (largest) | 1.09 (0.77-1.54) | 1.15 (0.78-1.70) |
| Arteriolar-to-venular ratio | | |
| 5 (largest) | 1.00 | 1.00 |
| 4 | 1.12 (0.79-1.59) | 0.98 (0.67-1.44) |
| 3 | 1.08 (0.77-1.53) | 1.02 (0.70-1.50) |
| 2 | 1.11 (0.78-1.59) | 0.96 (0.65-1.42) |
| 1 (smallest) | 1.45 (1.03-2.03) | 1.26 (0.87-1.83) |

* Model I: adjusted for age, gender and follow-up time
† Model II: additionally adjusted for smoking, body-mass index, total and high-density lipoprotein (HDL) cholesterol, intima-media thickness and systolic blood pressure

## DISCUSSION

Our main finding is that both retinal arteriolar and venular diameters were not related to the risk of iAMD. Only persons above age 75 years had a borderline increased risk with decreasing arteriolar diameters.

Strengths of the current study are its population-based prospective design, large study sample, and standardized procedures for both retinal vessel measurement and AMD diagnosis. Retinal vessel diameters were measured masked for the AMD grading and vice versa. Furthermore, because of the wide range of refractive errors in this population (range: –19.50 diopter (D) to +12.50 D; 97% between –6D to +6 D), we adjusted the diameters for possible magnification artefacts using Littmann's formula, thereby increasing the precision of the vessel measurements and enabling us to use the separate arteriolar and venular diameter sum values.

**Table 4**. Odds ratios (95% confidence interval) of age-related macular disease (AMD) adjusted for age, gender and follow-up time per standard deviation (SD) difference in retinal vessel diameters or arteriolar-to-venular ratio stratified on age

| | **Age < 75** | **Age ≥ 75** |
|---|---|---|
| N (cases) | 292 | 82 |
| Arteriolar narrowing* | 0.96 (0.85-1.09) | 1.30 (1.01-1.67) |
| Venular dilatation† | 1.08 (0.95-1.23) | 0.94 (0.75-1.18) |
| Arteriolar-to-venular ratio‡ | 1.04 (0.92-1.18) | 1.22 (0.96-1.56) |

* Per SD decrease in arteriolar diameters
† Per SD increase in venular diameters
‡ Per SD decrease in arteriolar-to-venular ratio

Also, some caution is warranted. We used the combination of early and late signs of AMD as incident outcome. Because participants with prevalent early AMD were excluded at baseline, late iAMD developed only in a small number of cases (n = 11) within the five-year follow-up period and hence the majority of incident cases were early iAMD. Given the well-documented risk of early AMD to progress to late AMD, it seems justified to assume that potential risk factors of early AMD also increase the risk of late AMD.[24] We did not have information on other retinal vessel signs (focal narrowing, arterio-venous nicking), because often only a limited part of the temporal arcade vasculature was visible on the images as sometimes was also the case in previous studies.[18] This prevented us from studying the effects of these determinants. Persons who participated in the follow-up examination but did not have optic disc transparencies for retinal vessel measurement were on average older and more often institutionalized, but were not different from the included participants with respect to their cardiovascular risk profile. Although, the differences between non-participants at follow-up and participants with optic disc transparencies were statistically significant for several cardiovascular risk factors, these were small suggesting a limited role for selective non-response.

Data from epidemiological studies are not consistent with respect to the causal relationship between cardiovascular risk factors such as blood pressure, markers of atherosclerosis and AMD. Some case-control studies reported an association,[6, 29, 30] whereas several cross-sectional population-based ones did not find one.[7, 8, 10, 17] The population-based Beaver Dam Study showed with increasing pulse pressure only a higher risk of incident retinal pigment epithelial (RPE) depigmentation (OR per 10 mm Hg increase: 1.17;

95% CI: 1.07-1.28) and exudative AMD (OR per 10 mm Hg increase: 1.34; 95% CI: 1.14-1.60). However, the relationships with systolic, diastolic blood pressure and other markers of cardiovascular disease were neither consistent nor strong.[5, 31] Recently, we reported a moderately increased risk of iAMD with increasing blood pressures (OR per 10 mm Hg increase in systolic blood pressure: 1.07; 95% CI: 1.01-1.13) and markers of atherosclerosis (OR per SD increase in carotid intima-media thickness: 1.15; 95% CI: 1.03-1.28).[9] It remains uncertain whether blood pressures measured at the arm are representative of local ocular perfusion.

Few studies looked at the relationship between retinal vascular abnormalities and AMD. Retinal arteriolosclerosis was reported to be associated with prevalent AMD.[17] However, after adjusting for age, this association disappeared. Another study reported borderline significant associations between retinal vascular abnormalities such as arterio-venous nicking (OR: 2.1; 95% CI: 0.9-4.9) and focal narrowing (OR: 2.2; 95% CI: 0.9-5.6) and iAMD, whereas retinal arteriolar diameters were not associated.[19] Recently, the Beaver Dam Eye Study also showed that retinal arteriolar diameters were not related to the 10-year incident early (OR: 1.1; 95% CI: 0.8-1.6) or late AMD (OR: 1.8; 95% CI: 0.6-5.4).[20] They suggested that these vascular retinal lesions reflected more severe vascular pathology than just the arteriolar diameters, which represented generalized narrowing. Blood velocity in the central retinal artery was reported to be impaired in clinic derived AMD patients compared to age-matched control subjects, mainly above the age of 75 years.[16]

For proper interpretation of these results some differences between the choroidal and retinal circulations should be mentioned. The choroidal circulation is held to be responsible for the nourishment of the RPE and the outer layers of the sensory retina (especially photoreceptors), and is characterized by a high blood flow and a low arterio-venous difference in oxygen content.[32] The retinal circulation in contrast is characterized by a low blood flow and high oxygen consumption.[32] Furthermore, apart from sympathetic and parasympathetic innervation there seems to be no auto-regulatory mechanisms to maintain choroidal blood flow unlike the retinal vasculature.[32, 33] The choroidal arteries are short, thereby transmitting the systemic blood pressure more directly to the choriocapillaris. Because of these differences the choroidal vessels might be more vulnerable to hypertensive damage than their retinal counterparts, as is also clinically known from disorders as pre-eclampsia or Elshnigs' spots in a hypertensive crisis.[33-35] These differences might explain the lack of an association between retinal vessels and iAMD in our overall analyses.

Arteriolar narrowing comprises on the one hand vasoconstriction (auto-regulation) and on the other hyalinosis, intimal thickening and medial hyperplasia.[33] The latter processes become increasingly important at older age.[22] Hence we divided our population into two age categories. We selected 75 years as a cut-off in order to secure enough cases in the oldest category. After stratification, smaller arteriolar diameters did predict AMD in participants 75 years and older. These retinal arterioles might be less able to cope with rising blood pressures with increasing age and the retinal arterioles may also be increasingly affected by hypertension and atherosclerosis, thus reflecting the status of choroidal vessels. An alternative explanation could be that the blood flow through the retinal vessels may decrease as a consequence of ganglion cell and nerve fiber atrophy following damage to cellular components in the outer sensory retina (photoreceptors) like we see in retinitis pigmentosa or central retinal artery occlusion.[36]

In conclusion, we did not find an association between retinal vessel diameters and iAMD in this general elderly population.

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# Chapter 3.3

# The Relationship between Refraction and Prevalent as well as Incident Age-related Maculopathy: The Rotterdam Study


## ABSTRACT

***Purpose*** – *To study the relationship between baseline spherical equivalents (SphE) of refraction and prevalent as well as incident age-related maculopathy (pARM and iARM).*

***Methods*** – *The study was performed as part of the Rotterdam Study, a population-based, prospective cohort study. The value for SphE (in diopters) measured with autorefraction and subjective optimization was taken of 6209 subjects aged 55 years and older. Aphakic or pseudophakic eyes at baseline were excluded. Stereoscopic transparencies of the macular region were graded according to the International Classification and Grading System. ARM was defined as large soft drusen with pigmentary changes, or indistinct drusen, or atrophic or neovascular age-related macular degeneration (AMD). For the prevalence analyses, we classified ARM into no, p(early)ARM or pAMD and per subject the eye with the most advanced ARM and the corresponding refraction were selected. After a mean 5.2 years of follow-up, 4935 subjects had complete data for our incidence analyses. Per subject the eye with iARM was selected.*

***Results*** – *The age- and gender-adjusted odds ratio (OR) of pARM (n = 536) for every diopter towards hyperopia was 1.09 (95% CI 1.04-1.13). For p(early)ARM (n = 440) the OR was 1.09 (1.04-1.14) and for pAMD (n = 96) the OR was 1.09 (1.00-1.19). Baseline refraction was significantly associated with increased risk of iARM (n = 497). Per diopter towards hyperopia the OR was 1.05 (95% CI 1.01-1.10). Additional adjustments for smoking, atherosclerosis and blood pressure did not alter the relationship.*

***Conclusions*** – *These population-based incidence data confirm results from prevalence and case-control studies that there is an association between hyperopia and ARM.*

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Age-related maculopathy (ARM) may be characterized by an accumulation of abnormal extracellular deposits (called drusen) in the vicinity of Bruch's membrane with or without pigmentary changes at the level of the retinal pigment epithelium (RPE).[1] Its end-stage, also called age-related macular degeneration (AMD), is the most important cause of incurable visual impairment in the Western World. Over the years, it has been proposed that these drusen are a manifestation of dysfunction and degeneration of the RPE and retina.[1] These age-related changes may also be a manifestation of restricted exchange of nutrients and other metabolic products between the neural retina across Bruch's membrane towards the choroid.

At the moment, there is limited long-term proven treatment for most AMD patients.[2-4] In a small number of subjects with neovascular AMD laser photocoagulation and photodynamic therapy can be successfully employed in delaying visual loss. Recently, the AREDS trial showed a protective effect of high-dose supplementation with antioxidant vitamins and zinc in a subset of ARM cases.[5] In view of preventive measures, better knowledge of pathophysiology and detection of early stages is important.[6]

Different studies have looked at various risk factors involved in

AMD, such as smoking, atherosclerosis, and genetic factors.[7] Likewise, it has been hypothesized that ocular factors, such as cataract (extraction), iris color and refractive errors might also be involved in the development of this disease.[8, 9] The association between hyperopia and AMD was first described by Maltzman et al. in 1979 in a case-control setting.[10] After that, a few other case-control studies on this topic showed conflicting results.[11-14] One report from a population-based cross-sectional study mentioned a weak association between hyperopia and early ARM (per diopter towards hyperopia: OR 1.1, 95% CI: 1.0-1.2).[15] More recently, a population-based follow-up study showed no relationship between refractive error and 10-year incidence of early ARM (hyperopia > 1.0 diopter versus emmetropia: RR 0.9, 95% CI 0.7-1.1) and AMD (RR 1.2, 95% CI 0.6-2.3).[16]

Since the nature of the association between refraction and ARM is still unclear, and may potentially provide insight into the pathogenesis of this disease, we examined the association between baseline refraction and prevalent (pARM) as well as incident ARM (iARM) in a population-based setting.

## METHODS AND MATERIALS

### Population

Information on the identification and description of the baseline study population has appeared in previous reports.[17] Briefly, the Rotterdam Study is a population-based prospective cohort study of the frequency and determinants of common cardiovascular, locomotor, neurological, and ophthalmologic diseases. The eligible population (n = 10,275) consisted of all inhabitants aged 55 years and older of a suburb of Rotterdam, the Netherlands. Of these, 7983 subjects (78%) agreed to participate in the study. Because the ophthalmologic part of the study became operational after the screening of participants had started, a smaller portion (n = 6780) participated in the ophthalmic examination. The study was conducted according to the tenets of the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline interviews and examinations were performed from 1990 to mid 1993, followed by a first follow-up examination from 1993 to 1994. A second follow-up screening took place from mid 1997 to the end of 1999.

**Diagnosis of age-related maculopathy**

A detailed description of the diagnostic procedures has been presented elsewhere.[18] Participants underwent a full eye examination, including stereo 35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) centered on field 2 (the fovea) following pharmacological mydriasis. The resulting transparencies were graded with 12.5x magnification according to the International Classification and Grading System for ARM and AMD.[19] In this system, all ARM fundus signs within a standard circle (diameter 6000 μm) around the fovea are recorded. Two graders, trained according to the Wisconsin ARM grading system and each having eight years experience, first graded the follow-up transparencies after which these were compared with those taken at baseline. The grading procedures and definitions, as well as the graders, were identical at baseline and at follow-up. Consensus sessions and between-grader comparisons were performed regularly. Weighted kappa values were 0.72 for soft distinct drusen, 0.80 for hyperpigmentation, and 0.58 for hypopigmentation.

ARM was defined as the presence of either large (≥63 μm) soft distinct drusen with pigmentary irregularities, or indistinct (≥125 μm) or reticular drusen, or atrophic or neovascular AMD. Atrophic AMD was defined as any sharply demarcated round or oval area of apparent absence of the RPE, larger than 175μm, irrespective of distance from the foveola but within the grid, with visible choroidal vessels and no neovascular AMD. Neovascular AMD was defined as the presence of a serous or hemorrhagic neuroretinal or RPE detachment and/or a subretinal neovascular membrane and/or a subretinal hemorrhage, and/or a periretinal fibrous scar. Lesions that were considered to be the result of generalized disease, such as diabetic retinopathy, chorioretinitis, high myopia, trauma, congenital diseases, or photocoagulation for reasons other than for neovascular AMD, were excluded from ARM classification.

**Refraction**

Refraction of each eye was taken as the mean of three measurements per eye with an autorefractometer (Topcon RM-2000, Topcon Corporation, Tokyo, Japan) followed by subjective optimisation. The medical doctors performing these measurements were trained by ophthalmologists and quality control sessions were organized routinely. In 2.5% of all subjects (1.9% of both eyes and 0.6% one eye) these autorefractometer measurements could not be obtained mostly due to lens opacities and physiological miosis. The value of their spectacles (if present) was taken as measured with a lensometer

(Topcon CL-1000, Topcon Corporation, Tokyo, Japan). Subjects with no autorefraction data and who did not have glasses were excluded from our analyses. For all analyses the spherical equivalents (SphE) were calculated and expressed in diopters.

**Study sample**

Of the 6780 participants in the ophthalmic part of the baseline study, 6477 (95.5%) persons underwent fundus photography and 6418 (94.7%) persons had gradable fundus transparencies in at least one eye. Prevalent ARM was diagnosed in 582 (9.1%) subjects, including 106 cases of AMD. This resulted in a cohort of 5836 subjects at risk who were free of ARM, i.e. subjects with no drusen, only hard drusen or soft drusen, or pigmentary abnormalities only. Of this cohort, 283 (4.8%) subjects died before the first follow-up examination and another 789 (13.5%) subjects died before the second follow-up. Of those alive at the first screening (n = 5553), 46 subjects were lost to follow-up, 905 refused to participate, and 13 had ungradable photographs. Of those alive at the second follow-up (n = 4764), 15 subjects were lost to follow-up, 1267 refused to participate, and 47 had ungradable photographs. In total, 4822 subjects (83% of those at risk) participated in at least one follow-up exam.

Of the 6418 (94.7%) subjects at baseline a total of 6209 (91.6%) subjects were included in the cross-sectional analysis, after excluding subjects with missing data on refraction and those who had had a cataract extraction in both eyes at baseline. From these subjects prevalent early ARM (p(early)ARM) was diagnosed in 440 cases and prevalent AMD (pAMD) in 96 cases (total prevalent ARM (pARM) n = 536). P(early)ARM was defined as pARM excluding pAMD.

In the follow-up analyses of incident ARM (iARM), all persons free of ARM at baseline and who participated at least in one follow-up exam were included (n = 4822). Furthermore, if the second eye of a pARM case was free of ARM, that eye was also included in this analysis, because we were looking at an eye-specific risk factor. Hence, 4935 (72.8%) subjects out of the 6209 participants at baseline on whom we had complete data were included for the follow-up analysis, resulting in 497 cases with iARM. Incidence of ARM was defined as absence ARM in an eye at baseline and presence of ARM in the same eye at follow-up. The mean follow-up time for the first examination was 2 years and for the second examination 6.5 years. The overall mean follow-up time was 5.2 years.

**Statistical analysis**

Analyses were started by taking the SphE in diopters as a continuous variable. Next, cut-off points were taken to define (advanced) myopia and (advanced) hyperopia. In the legends of table 1 the cut-off points are further specified. For categorized analyses emmetropic eyes were used as the reference group.

For cross-sectional analyses per subject the eyes were classified into no ARM, p(early)ARM or pAMD and subsequently the eye with most advanced ARM was chosen and the corresponding refraction of the same eye was included in the analyses. If both eyes had no ARM or the same pARM diagnosis, the right eye was chosen. However, if one eye was aphakic or pseudo-phakic and the other phakic, the latter was included. Subjects with bilateral cataract extraction at baseline were excluded from the analyses. The association between the SphE (in a continuous and a categorized way) and pARM was analyzed using logistic regression models adjusted for age and gender. To explore this relationship further we performed the analyses for p(early)ARM and pAMD separately.

For the incidence analyses, the eye that had iARM was selected and the corresponding refraction of that same eye at baseline was selected. In case both eyes developed iARM the right eye was chosen. Again logistic regression modeling was performed to establish the relationship of baseline

**Table 1.** Baseline characteristics and prevalence of early ARM and AMD per stratum of refraction. (Total n = 6209)

| | advanced myopia* | myopia† | emmetropia‡ | hyperopia§ | advanced hyperopia\|\| |
|---|---|---|---|---|---|
| Number | 443 | 770 | 966 | 3180 | 850 |
| Age (years) | 67.4 | 68.0 | 67.2 | 68.6 | 71.9 |
| Gender (% female) | 53.3 | 56.1 | 58.9 | 59.6 | 63.1 |
| Mean SphE (diopter) | - 5.5 | - 1.4 | 0.0 | + 1.6 | + 4.3 |
| p(early)ARM N (%) | 16 (3.6) | 57 (7.4) | 50 (5.2) | 227 (7.1) | 90 (10.6) |
| pAMD N (%) | 4 (0.9) | 10 (1.3) | 16 (1.7) | 43 (1.4) | 23 (2.7) |

* advanced myopia: SphE ≤ -3.0 D
† myopia: -3.0 D < SphE ≤ -0.5 D
‡ emmetropia: -0.5 D < SphE < +0.5 D
§ hyperopia: +0.5 D ≤ SphE < +3.0 D
|| advanced hyperopia: SphE ≥ +3.0 D

SphE with iARM correcting for age, gender and follow-up time. Follow-up time was calculated using the dates on which the baseline and follow-up photographs were made. Due to the low number of incident AMD (iAMD) cases, the analyses were not performed separately for incident early ARM (i(early)ARM) and iAMD. In multivariable models we further adjusted for smoking, atherosclerosis and blood pressure at baseline.

## RESULTS

Table 1 shows some general characteristics and the prevalence of ARM in our study population that was used for the cross-sectional analyses. In the figure the distribution of refraction at baseline is presented (mean SphE = + 0.83 D; Standard deviation = 2.6 D). Analyses with SphE as a continuous variable showed that every diopter towards hyperopia gave an age- and gender-adjusted odds ratio (OR) of 1.09 (95% Confidence Interval (CI): 1.04-1.13) for pARM (Table 2). Furthermore, when p(early)ARM and pAMD were studied separately, the risk estimates were the same for both. Every diopter towards hyperopia gave a significantly increased odds ratio for both p(early)ARM (OR: 1.09; 95% CI: 1.04-1.14) and pAMD (OR: 1.09; 95%

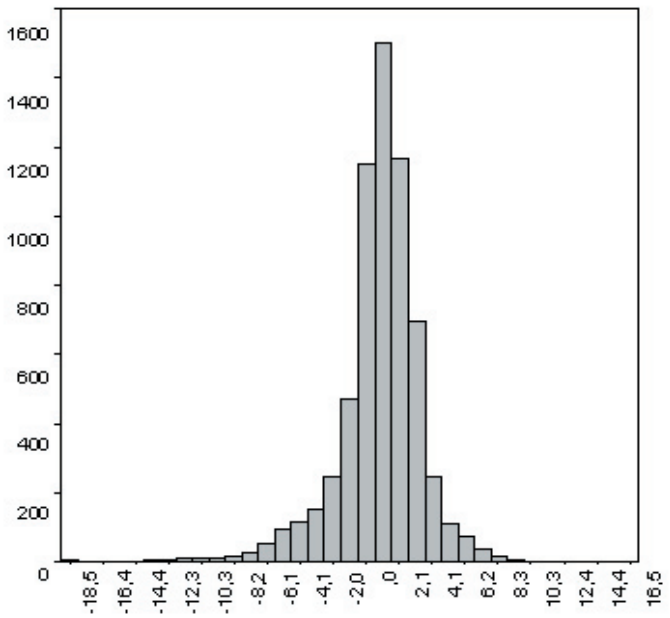


**Figure**. Distribution of refraction at baseline (n = 6209).

**Table 2**. Odds ratios (OR) of prevalent ARM according to spherical equivalents of refraction*

| | SphE (continuous)† OR (95% CI) | advanced myopia vs emmetropia‡ OR (95% CI) | myopia vs emmetropia‡ OR (95% CI) | hyperopia vs emmetropia‡ OR (95% CI) | advanced hyperopia vs emmetropia‡ OR (95% CI) |
|---|---|---|---|---|---|
| pARM (n = 536) | 1.09 (1.04-1.13) | 0.64 (0.38-1.09) | 1.21 (0.83-1.74) | 1.18 (0.89-1.58) | 1.46 (1.05-2.04) |
| p(early)ARM (n = 440) | 1.09 (1.04-1.14) | 0.67 (0.37-1.20) | 1.37 (0.92-2.05) | 1.32 (0.96-1.82) | 1.62 (1.12-2.34) |
| pAMD (n = 96) | 1.09 (1.00-1.19) | 0.61 (0.20-1.89) | 0.71 (0.31-1.64) | 0.87 (0.47-1.59) | 1.14 (0.57-2.24) |

* Adjusted for age and gender

† Range: -18.75 D to +15.13 D; OR per diopter towards hyperopia

‡ See legends table 1

**Table 3**. Incidence of ARM per stratum of refraction in absolute numbers. (n = 4935)

| | advanced myopia* | myopia* | emmetropia* | hyperopia* | advanced hyperopia* |
|---|---|---|---|---|---|
| Number | 372 | 622 | 778 | 2540 | 623 |
| iARM† N (%) | 26 (7.0) | 48 (7.7) | 73 (9.4) | 270 (10.6) | 80 (12.8) |

* see legends table 1

† Due to the low number of iAMD cases, they are not presented separately

CI: 1.00-1.19). Repeating the analyses with categorized SphEs (as defined in the legends of Table 1) showed that the risk of pARM was 46% higher for advanced hyperopia compared to emmetropia. In the categorized analyses, the association between advanced hyperopia and p(early)ARM (OR: 1.62; 95% CI 1.12-2.34) was still statistically significant, whereas the association disappeared for pAMD (OR: 1.14; 95% CI: 0.57-2.24). After additional adjustments for smoking, atherosclerosis and blood pressure, a statistically significant risk of pARM (OR: 1.07; 95% CI: 1.03-1.12) was found for every diopter towards hyperopia.

There were 497 subjects with iARM and table 3 shows the incidence of ARM stratified according to the refractive status of the eye. The same but attenuated results were found for the association between baseline refraction and iARM (table 4). After adjusting for age, gender and follow-up time, every diopter towards hyperopia increased the risk of iARM with 5% (OR: 1.05; 95% CI: 1.01-1.10). In the categorized analyses the risk estimates were not statistically significant, though there seemed to be a trend. After additional adjustments for smoking, atherosclerosis and blood pressure, the risk of iARM (OR: 1.04; 95% CI 1.00-1.09) for every diopter towards hyperopia remained statistically significant.

## DISCUSSION

Both the cross-sectional and to a lesser extent the follow-up results show that hyperopia is positively associated with ARM. For the proper interpretation of these findings we have to keep in mind several methodological aspects.

Subjects included in the present analyses differed from those excluded at baseline. These excluded subjects were not only those who did not participate in this study in the first place, but also those who were excluded because of missing data, ungradable photographs at baseline or bilateral

**Table 4**. Odds ratios (OR) of early incident ARM according to spherical equivalents of refraction*.

| | SphE (continuous)† OR (95% CI) | advanced myopia vs emmetropia‡ OR (95% CI) | myopia vs emmetropia‡ OR (95% CI) | hyperopia vs emmetropia‡ OR (95% CI) | advanced hyperopia vs emmetropia‡ OR (95% CI) |
|---|---|---|---|---|---|
| iARM (n = 497) | 1.05 (1.01-1.10) | 0.69 (0.43-1.11) | 0.79 (0.53-1.16) | 1.09 (0.82-1.43) | 1.20 (0.85-1.69) |

* Adjusted for age, gender and follow-up time
† Range: -18.75 D to +9.63 D; OR per diopter towards hyperopia
‡ See legends table 1

cataract extraction at baseline. Subjects who had had a bilateral cataract extraction were excluded, because we did not know the true refractive value of their natural lens and because of a potential relationship between cataract extraction and ARM.[9] In order to avoid misclassification of refractive status these subjects had to be excluded. The excluded subjects at baseline were on average older and more often institutionalized. Exclusion of this older cohort, that probably contained relatively more cases of ARM due to the older age distribution, may cause an imprecision in the estimate of the associations, leading to wider confidence intervals. However, we do not think that the point estimates (OR) were affected due to selection-bias by this exclusion. Although it is possible that having ARM causes non-participation, it is in our opinion unlikely that this non-participation was influenced by the refractive status.

This issue becomes even more important regarding the iARM analyses. As in any follow-up cohort, ours also showed that a relatively healthier population visited the research centre during the follow-up examinations, which again produced an imprecision of an underlying association, but probably not a bias.

Taking SphE as a continuous variable assumes that there is no biological difference between myopia and hyperopia. Because this assumption is not based on any empirical evidence and because there are many different ocular disorders associated with myopia versus hyperopia, we preferred to define myopia and hyperopia also using cut-off values with a group of emmetropic eyes as reference category. After doing so, we can still conclude that the relationship between ARM and hyperopia seems to be unaltered. Furthermore, the cut-off values for SphE were chosen after considering the distribution of refraction in this population. When the analyses were repeated using other cut-off values, we saw similar results (data not shown). It was

decided to take the presented cut-off values mainly in order to secure large enough numbers in all categories.

Our cross-sectional data have limitations when it comes to causal relationships. However, because nearly all previous studies have used cross-sectional data for this association, we also analyzed our p(early)ARM and pAMD cases in order to compare our results with other studies. One report from a population-based cross-sectional study mentioned a weak association between hyperopia and only early ARM (per diopter towards hyperopia; OR: 1.1, 95% CI: 1.0-1.2).[15] The case-control AREDS study showed an association between hyperopia and large drusen as well as with neovascular and atrophic AMD.[13] However, recently a population-based follow-up study showed no relationship between refractive status and 10-year incidence of ARM.[16] On the basis of our cross-sectional analyses we can confirm that there seems to be a relationship between SphE and p(early)ARM as well as pAMD. Furthermore, our results from the follow-up analyses support the hypothesis that there might be a causal relationship. In our attempt to fully explore the relationship between refraction and i(early)ARM as well as iAMD separately we were, however, limited by the small number of iAMD cases.

Also, diagnostic procedures need to be considered. Drusen in myopic fundi may be more difficult to assess due to the usually lower RPE and choroidal pigmentation resulting in a blonder fundus and less contrast. Another diagnostic pitfall might be that during the grading of the fundus transparencies no Littmann's correction was used for the variation in magnification caused by the refractive state of that eye. A photograph taken of a hyperopic eye will lead to a larger magnification due to the hyperopia compared to a myopic (or even an emmetropic) eye. This could have introduced a misclassification into our grading of drusen and might play a role to a certain extent in the cross-sectional analyses. In the follow-up analyses, however, the incident cases were defined as a change in ARM stage and the absolute dimensions of the drusen per se were not important. Thus, here the magnification should pose fewer problems. There could finally be a tendency to classify a "neovascular AMD" eye in a myopic fundus with a few or no drusen as a myopic disciform reaction or Fuch's spot instead of neovascular AMD. This could (partly) account for the association found between hyperopia and neovascular pAMD (per diopter towards hyperopia: OR: 1.21, 95% CI: 1.09-1.36).

Additional adjustments for other known risk factors such as smoking, blood pressure and atherosclerosis did not significantly alter this relationship, showing that refraction has an additional effect on the development of ARM. At baseline 13.4% of participants used any type of micronutrient supplements,

but we did not have any information on the exact dose, type and duration of use. Moreover, we are unaware of an association between micronutrient use and refraction, therefore we did not adjust for this use.

The pathophysiological mechanism by which hyperopia may lead to ARM still remains to be elucidated. We think that of the three components determining the refraction of an eye, corneal curvature, lens power and axial length, the latter one is most likely to play a role in the pathogenesis of ARM. In general, hyperopic eyes are smaller and have thicker as well as more rigid and compact sclerae.[20] This generalized stiffness of the sclera may cause an increase in resistance of the choroidal venous outflow with throttling of the vorticose veins,[21, 22] and a thicker choroid.[23, 24] Both histologic and in vivo studies with laser Doppler flow measurements have shown an increased choroidal resistance in AMD-cases compared to gender- and age-matched controls.[25] We speculate that decreased flow prevents easy exchange of nutrients and metabolic products across the RPE and results in drusen formation and thickening of Bruch's membrane. However, the exact role of these vascular abnormalities in the pathogenesis of ARM remains unclear.[26]

Other speculations that may explain the observed association with hyperopia may be that a poorer cooling of the retina by impaired choroidal blood flow leads to a higher susceptibility for oxidative stress. Also the thicker retina in hyperopic versus myopic eyes may have a higher need for oxygen and nutrients. Furthermore, it is uncertain whether the photoreceptor density per $mm^2$ of the fovea or per RPE cell is different in hyperopic subjects.

Although the magnitude of the risk estimates associated with refraction is lower than that of the major well-known risk factors such as age and smoking,[7] the risk of 5-9% per diopter towards hyperopia is still a considerable increase. In view of these findings, special attention might have to be given to older persons with hyperopia (e.g. over 70 years with hyperopia > 3.0 diopter) in order to offer them in the presence of ARM the potential benefit from micronutrient supplementation.

In conclusion, this large population-based cohort study showed also in a prospective way that hyperopia is a risk factor for ARM.

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# Chapter 3.4

# Retinal Vessel Diameters and Incident Open-Angle Glaucoma and Optic Disc Changes: The Rotterdam Study


## ABSTRACT

***Purpose*** *– It remains unclear whether reduced retinal blood flow and smaller arterioles, reported to exist in patients with open-angle glaucoma (OAG), are a cause or a consequence of ganglion cell loss. We examined whether baseline retinal vessel diameters were related to incident OAG (iOAG) or incident optic disc changes in a population-based setting.*

***Methods*** *– In the prospective population-based Rotterdam Study, baseline diameters of retinal arterioles and venules (1990-1993) were measured on digitized images of 3469 persons (≥ 55 years) at risk for OAG. The follow-up examinations took place from 1997 to 1999. Incident OAG was based on the presence of incident glaucomatous visual field loss and/or incident glaucomatous optic neuropathy. Changes in neuro-retinal rim, cup area or vertical cup-to-disc ratio were calculated with the semi-automated ImageNet analyzer in 2782 persons.*

***Results*** *– After a mean follow-up time of 6.5 years, 74 participants developed iOAG. At baseline the mean arteriolar diameter was 147.5µm (standard deviation (SD): 14.2) and the venular 222.9µm (SD: 20.0). Neither arteriolar diameters (odds ratio (OR) per SD decrease: 0.82; 95% confidence interval (CI): 0.66-1.03) nor venular ones (OR per SD increase: 1.20; 95% CI: 0.95-1.53) were significantly related to iOAG. Baseline retinal vessel diameters did not predict changes in the optic disc. Additional adjustment for cardiovascular risk factors did not alter these results.*

***Conclusions*** *– Our data show that baseline retinal vessel diameters did not influence the risk of iOAG or incident optic disc changes. These data provide no evidence for a retinal vascular cause in the pathogenesis of OAG.*

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Open-angle glaucoma (OAG) is characterized by progressive loss of retinal ganglion cells and their axons, resulting in glaucomatous optic neuropathy (GON) with corresponding glaucomatous visual field loss (GVFL). Despite being the second leading cause of incurable visual impairment in the Western world, little is known about the etiology of OAG.[1, 2] Age,[1] intra-ocular pressure (IOP),[3] myopia,[4] African origin,[5] and family history[6] are some of the factors associated with OAG.

Other possible risk factors are those related to the perfusion of the optic nerve head or the retinal ganglion cell layer.[7-9] Data concerning the relationship between vascular risk factors and OAG remain controversial,[9] as is the association between blood pressure measured at the brachial artery and prevalent OAG.[10, 11] Peripheral vascular markers might not represent local vascular abnormalities in OAG patients. To overcome this problem, studies have used blood flow measurements in the ophthalmic and posterior ciliary arteries in patients with OAG,[12-16] showing 10-20% decreased ocular blood flow compared to age-matched controls.[16, 17] Some of these cross-sectional and case-control studies used small numbers or highly selected clinic-based patients. To our knowledge, prospective data on the relationship between retinal vascular factors and incident OAG (iOAG) are lacking. Due to these

limitations an important etiological question remains unanswered: whether an impaired retinal circulation plays a causative role in the pathophysiology of OAG.[9]

Recently, a semi-automated system was developed to measure retinal vessel diameters.[18] We reported that smaller arteriolar diameters were associated with higher blood pressures, and larger venular diameters with atherosclerosis and inflammation.[19] We tested in the present study the hypothesis that smaller arteriolar or larger venular diameters at baseline increased the risk of iOAG in a prospective population-based cohort. Furthermore, we investigated whether these diameters were related to incident optic disc changes.

## PARTICIPANTS AND METHODS

### Study population

The present study was part of the Rotterdam Study, a population-based, cohort study on chronic diseases in the elderly.[1, 20] A total of 7983 persons aged 55 years and older living in a district of Rotterdam agreed to participate. Because the ophthalmic part became operational after the screening of at random invited participants had started, a total of 6780 participants underwent the ophthalmic examination.[1] The study was conducted according to the Declaration of Helsinki, and the Medical Ethics Committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline home interviews and examinations were performed from 1990 to mid-1993. The follow-up examinations took place from mid-1997 to the end of 1999.

The ophthalmologic examination, both at baseline and follow-up, comprised Goldmann applanation IOP,[21] visual field (VF) testing and after pharmacological mydriasis direct and indirect ophthalmoscopy as well as simultaneous stereoscopic fundus photography of the optic disc on both eyes with a telecentric fundus camera ($20^0$ field, Topcon Optical Company, Tokyo, Japan).[1]

### Retinal vessel measurements

After digitizing the optic disc photographs with a high-resolution scanner, per participant the digitized image with the best quality (left or right eye) was analyzed with a semi-automated system (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison) by four trained graders masked for the endpoints.[19] We used the improved Parr-Hubbard formula to compute the summary arteriolar

and venular diameters.[22] Because eyes may have a different magnification due to refractive errors we additionally adjusted these summary vessel measures for the refraction using Littmann's formula to obtain corrected measures.[23] The arteriolar-to-venular ratio (AVR) was taken as the ratio of the arteriolar to venular diameters.

**Optic disc morphometry**

The above-mentioned optic disc transparencies were also analyzed independently from the retinal vessel measurements with the semi-automated ImageNet analyzer (Topcon ImageNet; Topcon Optical Company, Tokyo, Japan) in order to calculate the areas (in $mm^2$) of the optic disc, cup and neuro-retinal rim, the minimal rim width and the vertical cup-to-disc ratio (VCDR).[24] The system's hardware, its software modules and reproducibility of measurements have been described.[25-27] The same eye was taken for both the retinal vessel diameters and optic disc measurements.

**Glaucoma diagnosis**

We considered OAG to be present in persons who had at least in one and the same eye an open anterior chamber angle, no history or signs of angle closure or secondary glaucoma, and the presence of GON and/or GVFL.[1]

We defined GON using measurements obtained with ImageNet, whenever available.[1] Possible GON was defined as a VCDR $\geq$ 0.7, or asymmetry between eyes $\geq$ 0.2, or minimal rim width $<$ 0.10. Probable GON was defined as a VCDR $\geq$ 0.8, or asymmetry between eyes $\geq$ 0.3, or minimal rim width $<$ 0.05. When ImageNet data were absent, funduscopic VCDR was used leading to a slightly different definition of probable GON based on the distribution in the population: instead of VCDR $\geq$ 0.8, it was VCDR $\geq$ 0.9.[1] Minimal rims were not assessed funduscopically, and were in these cases not taken into account.

The VF of each eye separately was screened with a 52-point supra-threshold test that covered the central field with a radius of $24^0$. If the test was unreliable, or a reliable test showed VFL in at least one eye, this test was repeated on that eye. When the second test again was unreliable, or VFL was still present, Goldmann kinetic perimetry was performed on both eyes.[28] Glaucomatous VFL was defined as VFL compatible with OAG after exclusion of all other (neuro-)ophthalmic causes. Definite OAG was defined as presence of GVFL in combination with possible or probable GON. Probable OAG was either presence of GVFL in the absence of GON or the

presence of probable GON in the absence of GVFL.[1]

Incidence of OAG was defined as having no OAG in both eyes at baseline and acquiring probable or definite OAG in at least one eye at follow-up. Excluded from this incidence definition were those who had possible GON at baseline and developed probable GON at follow-up, because this increase may be quite small and due to variability in measurement of GON.

**Assessment of confounders**

The average of two blood pressure measurements in sitting position at the right brachial artery with a random-zero sphygmomanometer was taken. Mean perfusion pressure was calculated using the following equation: (2/3*diastolic blood pressure + 1/3*systolic blood pressure – IOP). Non-fasting serum total cholesterol was determined by an enzymatic procedure and high-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[29] The ratio of the total to HDL cholesterol was taken. Diabetes mellitus was considered present if participants reported use of antidiabetic medication or when random or post-load serum glucose level was > 11 mmol/l.[30] Intima-media thickness was measured in the common carotid arteries using ultra-sonography.[31] Information on smoking (categorized as current, former or never) was obtained during the baseline home interview.

**Study sample**

Of the 6780 participants in the ophthalmic part of the Rotterdam Study, 6436 persons had optic disc photographs and in 5674 persons fundus transparencies were gradable for retinal vessel measurements. Excluding 157 persons with prevalent OAG, a total of 5517 participants were at risk for iOAG. During follow-up 838 participants died, a further 1210 refused or were unable to participate at the follow-up examination leaving a total of 3469 participants for the current analyses.

Also, 2782 participants had gradable stereo disc transparencies taken both at baseline and follow-up in at least one and the same eye in order to calculate changes in optic disc morphometry with ImageNet.

**Data analyses**

In order to assess whether participants were different from non-participants or those who died, mean differences were calculated for the continuous variables using analysis of covariance and odds ratios (OR) for the categorical ones using logistic regression models. The mean differences and the ORs were adjusted for age and gender.

Odds ratios with corresponding 95% confidence intervals (CI) for iOAG were calculated by analyzing retinal vessel diameters both linearly (per standard deviation (SD)) and in quartiles, adjusted for age, gender and follow-up time, and additionally for other known cardiovascular risk factors. For additional adjustments we included those cardiovascular risk factors that we previously showed to be associated with retinal vessel diameters,[19] and that also have been implicated in the pathogenesis of glaucoma.[9] Although IOP is an important risk factor for glaucoma, IOP was not included for additional adjustment because it was not related to the retinal vessel diameters. Quartiles were selected to secure enough cases in each category.

Because, retinal vessel measurements were performed in the eye with the best quality fundus transperancy, it could happen that the vessel measurement was done in one eye, whereas iOAG developed in the other. In these cases vessels were measured in the eye with iOAG in order to do an eye-specific sub-analysis.

Incident optic disc changes were defined as the difference between follow-up and baseline measurements in neuro-retinal rim area, cup area, or the VCDR. Analysis of covariance models were used to compute the age- and gender-adjusted mean changes in neuro-retinal rim area, cup area and VCDR for the different quartiles of retinal vessel diameters. Statistical significance level was set at a p-value of 0.05. We used SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

Table 1 shows the baseline characteristics of the study population. After a mean of 6.5 years of follow-up (range: 5.2-9.4) a total of 74 participants developed iOAG, of whom 52 had probable and 22 definite iOAG. In persons with iOAG the mean arteriolar diameter at baseline was 149.8µm (SD: 14.0), venular 225.6µm (SD: 17.4) and AVR 0.67 (SD: 0.06), and in those without iOAG these mean values were 147.5µm (SD: 14.2), 222.9µm (SD: 20.1) and 0.66 (SD: 0.06), respectively. Table 2 shows that neither retinal arteriolar, nor venular diameters, nor the AVR were related to the risk of iOAG. Categorizing retinal vessel diameters into quartiles did not show a consistent trend towards a higher risk of iOAG (Table 3). Additional adjustments for other cardiovascular risk factors did not affect these results.

In the 74 persons with iOAG, 8 had bilateral iOAG. In 40 cases with iOAG the vessel measurements were already performed on the eye with iOAG. For an eye-specific analysis we also measured the retinal vessels in

**Table 1**. Baseline characteristics

| | Participants | Non-participants* | Adjusted† differences‡ (95% CI) | Died§ | Adjusted† differences \|\| (95% CI) |
|---|---|---|---|---|---|
| Number | 3469 | 1210 | | 838 | |
| Age (years) | 65.4 (6.6) | 70.1 (8.2) | 4.7 (4.2; 5.2)** | 75.1 (8.6) | 9.8 (9.3; 10.4)** |
| Gender (%, female) | 57.6 | 67.9 | 1.41 (1.23; 1.63)** | 51.0 | 0.62 (0.52; 0.73)** |
| Diabetes mellitus (%) | 6.5 | 10.2 | 1.38 (1.08; 1.75)** | 21.4 | 2.78 (2.18; 3.56)** |
| Smoking (%), Current | 21.9 | 25.6 | 1.68 (1.43; 1.98)** | 28.2 | 2.38 (1.95; 2.90)** |
| Past | 45.6 | 38.1 | 0.82 (0.71; 0.95)** | 39.2 | 0.65 (0.54; 0.78)** |
| Diastolic blood pressure (mmHg) | 73.6 (10.8) | 74.3 (11.7) | 1.6 (0.8; 2.3)** | 73.2 (12.8) | 0.9 (-0.1; 1.8) |
| Systolic blood pressure (mmHg) | 135.6 (20.7) | 142.0 (22.7) | 3.6 (2.1; 5.0)** | 144.9 (24.3) | 3.4 (1.6; 5.2)** |
| Carotid intima-media thickness (mm) | 0.77 (0.14) | 0.80 (0.15) | 0.01 (0.00; 0.02)** | 0.87 (0.17) | 0.04 (0.02; 0.05)** |
| Serum total cholesterol (mmol/l) | 6.68 (1.17) | 6.71 (1.19) | 0.04 (-0.04; 0.12) | 6.34 (1.28) | -0.17 (-0.27; -0.07)** |
| Serum HDL# cholesterol (mmol/l) | 1.36 (0.35) | 1.38 (0.36) | 0.01 (-0.02; 0.03) | 1.28 (0.38) | -0.04 (-0.06; -0.01)** |
| Intra-ocular pressure (mmHg) | 15.5 (2.97) | 15.6 (3.10) | 0.14 (-0.07; 0.35) | 15.3 (3.21) | -0.12 (-0.38; 0.13) |
| Retinal arteriolar diameters (µm) | 147.5 (14.2) | 146.8 (14.5) | 0.2 (-0.8; 1.2) | 144.7 (14.8) | -0.8 (-2.0; 0.4) |
| Retinal venular diameters (µm) | 222.9 (20.0) | 220.8 (21.1) | 0.6 (-0.7; 2.0) | 219.8 (22.9) | 1.4 (-0.3; 3.1) |
| Arteriolar-to-venular ratio | 0.66 (0.06) | 0.67 (0.06) | -0.001 (-0.005; 0.003) | 0.66 (0.06) | -0.007 (-0.012; -0.002)** |

Presented as Unadjusted Means (Standard Deviation) or Percentages. Adjusted Differences are Presented as Mean Differences for Continuous and Odds Ratios for Categorical Variables with 95% confidence intervals (CI)

* Unable or refused at follow-up
† Age and gender adjusted if applicable
‡ Non-participants versus Participants
§ Persons who died before the follow-up examination
|| Deceased persons versus Participants
# HDL = high-density lipoprotein
** Significant ($p < 0.05$) compared to participants

**Table 2**. Odds ratios* of incident open-angle glaucoma per standard deviation difference in baseline retinal vessel diameters (n = 3469)

| | Model I\|\| | Model II# |
|---|---|---|
| Generalized arteriolar narrowing† | 0.82 (0.66-1.03) | 0.91 (0.70-1.17) |
| Generalized venular dilatation‡ | 1.20 (0.95-1.53) | 1.15 (0.89-1.49) |
| Arteriolar-to-venular ratio§ | 0.98 (0.77-1.24) | 1.05 (0.81-1.37) |

* Odds ratios (OR) with corresponding 95% confidence interval
† OR per standard deviation decrease in retinal arteriolar diameters
‡ OR per standard deviation increase in retinal venular diameters
§ OR per standard deviation decrease in arteriolar-to-venular ratio
|| Model I: adjusted for age, gender, and follow-up time
# Model II: additionally adjusted for diabetes mellitus, smoking, total to HDL cholesterol ratio, mean perfusion pressure, and intima-media thickness

26 of the remaining 34 persons who had gradable images in the same eye in which iOAG was diagnosed. In these 66 cases, neither arteriolar narrowing (OR: 0.84; 95% CI: 0.66-1.07), nor venular dilatation (OR: 1.16; 95% CI: 0.92-1.48), nor AVR (OR: 0.96; 95% CI: 0.76-1.23) were related to iOAG.

The mean area of the neuro-retinal rim at baseline was 1.81 $mm^2$ (range: 0.79-3.98; SD: 0.37), of the cup 0.58 $mm^2$ (range: 0.02-2.11; SD: 0.34), and the mean VCDR 0.49 (range: 0.04-0.78; SD: 0.13). At follow-up, the mean change in area for the neuro-retinal rim was 0.029 $mm^2$ (SD: 0.21), for the cup 0.021 $mm^2$ (SD: 0.16), and for VCDR 0.004 (SD: 0.07). Table 4 presents the relationship between quartiles of retinal vessel diameters and the change in these optic disc parameters. Baseline retinal vessel diameters were not related to incident optic disc changes.

## DISCUSSION

In this prospective study in community-dwelling elderly people, our main finding was that both retinal arteriolar and venular diameters at baseline were not related to an increased risk of OAG. In line with these observations, the retinal vessel diameters did not predict incident optic disc changes.

A potential limitation of our study is the number of non-participants at follow-up. The large number of deaths during follow-up is due to the fact that our cohort was composed of elderly persons. If persons who died before the follow-up examination had developed OAG prior to their death more often than those who survived, this would have biased the results towards the null value. However, we have previously shown that people who have OAG

are subsequently not at an increased risk of death excluding the possibility that survival bias explained our negative findings.[32] Furthermore, persons who died before the follow-up examination and those who refused to participate showed statistically significant differences compared to the participants with respect to their cardiovascular profile, but the retinal vessel diameters were not different, suggesting a limited role for selective non-response. This loss to follow-up probably resulted in imprecision of an underlying association leading to larger confidence intervals. Hence, we cannot rule out the possibility that we were unable to detect small effects due to the small number of incident cases. Another limitation was that photographs were not taken synchronized on the cardiac cycle, leading to variation in vessel diameter due to pulsatility.[33] However, because photography was independent of any participant characteristics, this will have caused random misclassification.

**Table 3**. Odds ratios***** of incident open-angle glaucoma in quartiles of baseline retinal vessel diameters (n = 3469)

| | **Model I†** | **Model II‡** |
|---|---|---|
| Retinal arteriolar diameters | | |
| 4 (largest) | 1.0 (Reference) | 1.0 (Reference) |
| 3 | 0.72 (0.39-1.35) | 0.75 (0.38-1.48) |
| 2 | 0.61 (0.32-1.18) | 0.69 (0.34-1.40) |
| 1 (smallest) | 0.70 (0.39-1.35) | 0.91 (0.46-1.79) |
| Retinal venular diameters | | |
| 1 (smallest) | 1.0 (Reference) | 1.0 (Reference) |
| 2 | 2.17 (1.04-4.52) | 1.81 (0.85-3.85) |
| 3 | 2.23 (1.07-4.66) | 1.98 (0.93-4.20) |
| 4 (largest) | 1.98 (0.93-4.22) | 1.48 (0.66-3.31) |
| Arteriolar-to-venular ratio | | |
| 4 (largest) | 1.0 (Reference) | 1.0 (Reference) |
| 3 | 1.47 (0.78-2.76) | 1.66 (0.84-3.28) |
| 2 | 1.24 (0.64-2.39) | 1.45 (0.71-2.95) |
| 1 (smallest) | 0.78 (0.38-1.63) | 0.92 (0.41-2.07) |

* Odds ratios (OR) with corresponding 95% confidence interval
† Model I: adjusted for age, gender, and follow-up time
‡ Model II: additionally adjusted for diabetes mellitus, smoking, total to HDL cholesterol ratio, mean perfusion pressure, and intima-media thickness

**Table 4**. Mean change* of optic disc dimensions in quartiles of baseline retinal vessel diameters (n = 2782)

| | Cup area ($mm^2$) | Rim area ($mm^2$) | VCDR† |
|---|---|---|---|
| Retinal arteriolar diameters | | | |
| 4 (largest) | 0.030 (0.018; 0.042) | 0.024 (0.008; 0.039) | 0.005 (0.000; 0.010) |
| 3 | 0.014 (0.002; 0.026) | 0.028 (0.013; 0.043) | 0.003 (-0.002; 0.008) |
| 2 | 0.019 (0.007; 0.031) | 0.027 (0.012; 0.043) | 0.005 (0.000; 0.009) |
| 1 (smallest) | 0.021 (0.009; 0.033) | 0.037 (0.022; 0.052) | 0.005 (0.001; 0.010) |
| Test for trend | p = 0.33 | p = 0.67 | p = 0.97 |
| Retinal venular diameters | | | |
| 1 (smallest) | 0.024 (0.012; 0.036) | 0.032 (0.016; 0.047) | 0.005 (0.000; 0.010) |
| 2 | 0.016 (0.003; 0.027) | 0.034 (0.019; 0.050) | 0.003 (-0.002; 0.008) |
| 3 | 0.013 (0.001; 0.024) | 0.034 (0.019; 0.050) | 0.001 (-0.004; 0.006) |
| 4 (largest) | 0.033 (0.021; 0.045) | 0.016 (0.000; 0.031) | 0.008 (0.003; 0.013) |
| Test for trend | p = 0.08 | p = 0.28 | p = 0.25 |
| Arteriolar-to-venular ratio | | | |
| 4 (largest) | 0.024 (0.012; 0.036) | 0.026 (0.011; 0.042) | 0.004 (-0.001; 0.009) |
| 3 | 0.013 (0.001; 0.025) | 0.036 (0.020; 0.051) | 0.002 (-0.003; 0.007) |
| 2 | 0.018 (0.007; 0.030) | 0.031 (0.016; 0.046) | 0.004 (-0.001; 0.009) |
| 1 (smallest) | 0.029 (0.017; 0.041) | 0.023 (0.008; 0.039) | 0.008 (0.003; 0.013) |
| Test for trend | p = 0.29 | p = 0.70 | p = 0.50 |

* Age and gender adjusted mean changes with corresponding 95% confidence intervals
† VCDR = vertical cup-to-disc ratio

Strengths of the present study are its prospective population-based design, a large number of community-dwelling elderly persons, accurate and objective quantification of retinal vessel diameters and standardized definitions for iOAG.

In systemic hypertension, the increased peripheral vascular resistance may impair ocular perfusion.[34] In the Rotterdam Study, blood pressure was associated with prevalent high-tension OAG (OR per SD increase in pulse pressure: 1.32; 95% CI: 1.03-1.69), but not with prevalent normal-tension OAG (OR: 0.97; 95% CI: 0.82-1.15) (Hulsman et al, personal communication). The increased risk of high-tension OAG was partly due to the positive correlation between blood pressure and IOP.[7, 11] However, it remains unclear

whether high blood pressure, independent of its effect on IOP, is related to OAG.[34] No such relationship was established in either the Barbados Eye Study (OR: 1.29; 95% CI: 0.65-2.59),[11] or the Baltimore Eye Survey (OR: 1.32; 95% CI: 0.60-2.92).[7] Alternatively, hypotension rather than hypertension has been proposed to be deleterious to the optic nerve function.[9, 34] A decreased diastolic perfusion pressure was related to prevalent OAG (OR: 3.29; 95% CI: 2.06-5.28).[11] It remains a point, however, to what extent systemic blood pressures are representative of the local perfusion of the optic nerve head and the retinal ganglion cell layer.[11]

Few studies, thus far, looked at the relationship between retinal vessel abnormalities and OAG. One study showed that OAG patients had significantly smaller arteriolar diameters (n = 281; mean: 91μm; SD: 20), measured on optic disc photographs, than age-matched control persons (n = 173; mean: 104μm; SD: 18).[12] In a population-based cross-sectional study, prevalent OAG cases (n = 59; mean: 183μm) also had smaller arteriolar diameters compared to controls (n = 3065; mean: 194μm).[35] Conversely, in another study using digital scanning laser fluorescein angiography, no differences for either arteriolar or venular diameters were observed in OAG patients compared to control persons, although retinal arteriovenous circulation time was substantially prolonged.[36] Clinically, it is also known that reduced retinal blood perfusion, such as in central retinal artery occlusion or non-arteritic anterior ischemic optic neuropathy, often does not lead to glaucomatous cupping.[14, 37] Our prospective data provide evidence against a retinal vascular cause in the pathogenesis of retinal ganglion cell loss, and the subsequent development of GVFL. Only in the categorized analysis, it seemed that larger venular diameters were related to iOAG. However, there was no clear trend and this association disappeared after additional adjustments. The results from the linear models for iOAG (table 2) and the models for optic disc changes (table 4) also supported the view that this was a spurious finding.

For proper interpretation of these results local differences in ocular circulation need to be discussed.[9, 38] The inner part of the retina (including the retinal ganglion cell layer) and the surface layer of the optic nerve head are vascularized by the retinal arterioles, whereas the main sources of blood supply to the optic nerve head are the short posterior ciliary arteries, either directly or from the circle of Haller and Zinn.[38] It has been reported that the auto-regulation in the short posterior ciliary arteries seems to be less efficient than in the retinal circulation.[9] Also, in contrast to the retinal vessels, the optic nerve head vasculature has no proper blood-tissue barrier, making it more sensitive to fluctuating levels of vaso-active molecules (such as angiotensin-

II).[9] Because of these differences the short posterior ciliary arteries may be more vulnerable to vascular damage than the retinal vessels. This is supported by several studies suggesting that in OAG impairment in blood flow was more prominent in the short posterior ciliary arteries than in the retinal arteries.[13, 15, 39]

Animal models have also suggested that the retinal circulation may not be causally related to OAG.[40] Administration of endothelin-1, a vaso-constrictive agent that reduces among others the retinal blood flow, resulted in loss of retinal ganglion cells and their axons in rats,[40] probably mediated by apoptosis.[41] However, this type of vascular injury did not lead to optic disc cupping.[40] It has been suggested that remodeling of the extra-cellular matrix, irrespective of the origin of retinal ganglion cell loss, is the hallmark of optic disc cupping.[40] Activation of quiescent astrocytes (for example by an increase in IOP) could lead to an increased expression of metalloproteinases, enzymes that play an important role in remodeling the optic disc and eventually leading to cupping.[42]

In conclusion, we have shown that baseline retinal vessel diameters did not increase the risk of iOAG or incident glaucomatous optic disc changes. The results reported here provide no evidence for a retinal vascular role in the pathogenesis of OAG. Further prospective studies need to be conducted to confirm these findings and to elucidate the possible role of vascular factors in the patho-physiology of OAG.

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# *Part IV*

# *Neuro-epidemiology*

# Chapter 4.1

# Retinal Vessel Diameters and Risk of Stroke: The Rotterdam Study


## ABSTRACT

***Background*** – *Retinal vessels may provide information on vascular pathology of the brain, because they share many features with cerebral vessels. A smaller retinal arteriolar-to-venular ratio reportedly predicts the risk of stroke. It is unclear if this is due to arteriolar narrowing or venular dilatation. We investigated whether smaller arteriolar or larger venular diameters were related to the risk of stroke and cerebral infarction.*

***Methods and Results*** – *This study was based on the prospective population-based Rotterdam Study (1990-1993) and included 5540 participants of 55 years or over, who had gradable fundus transparencies and were free of stroke at baseline. For each participant, retinal arteriolar and venular diameters were measured on digitized images of one eye. Follow-up for first-ever stroke was complete until January 1, 2002.*
*After a mean follow-up of 8.5 years, 411 participants developed a stroke, of whom 259 had cerebral infarction. Larger venular diameters were associated with an increased risk of stroke (hazard ratio (HR) adjusted for age and gender per standard deviation (SD) increase: 1.12 [95% confidence interval (CI): 1.02-1.24]) and cerebral infarction (HR: 1.15 [95% CI: 1.02-1.29]). Smaller arteriolar diameters were neither related to the risk of stroke (HR per SD decrease: 1.02 [95% CI: 0.93-1.13]), nor to the risk of cerebral infarction (HR: 1.02 [95% CI: 0.90-1.15]). After additional adjustment for other cardiovascular risk factors, the results did not alter.*

***Conclusions*** – *Larger retinal venular diameters are associated with an increased risk of stroke and cerebral infarction. The role of venules in cerebrovascular disease needs further exploration.*

Submitted

In the last few decades most epidemiological studies addressed the role of vascular pathology in the pathogenesis of stroke by studying classical cardiovascular risk factors such as systemic blood pressure and markers of sub-clinical atherosclerosis.[1-3] However, these non-invasive markers of vascular pathology are related to blood vessels outside the brain and may not reflect local cerebrovascular abnormalities. Furthermore, the cerebral circulation is difficult to assess. Retinal vessels have been suggested to provide information on vascular pathology of the brain, because they share many features with cerebral vessels such as blood-tissue barrier, auto-regulation and embryological origin.[4,5]

Recently, a semi-automated system was developed to measure the retinal arteriolar and venular diameters.[4,6] A lower arteriolar-to-venular ratio (AVR) thus obtained was suggested to reflect generalized arteriolar narrowing and was associated with an increased risk of stroke.[7,8] The AVR does not specify whether this association is due to generalized arteriolar narrowing, venular dilatation or both. We recently showed that the assumption that venular diameters do not vary with age and other vascular risk factors is no longer tenable.[9] Smaller arterioles are related to higher blood pressures and larger venules to atherosclerosis, markers of inflammation and high-density

lipoprotein (HDL) cholesterol levels.[9] To our knowledge, there are no reports on the association between arteriolar and venular diameters separately and the risk of stroke.

We studied the association between retinal arteriolar and venular diameters and the risk of first-ever incident stroke or its major subtype cerebral infarction in a prospective population-based cohort.

## METHODS

### Population

The present study was performed as part of the Rotterdam Study, a population-based, cohort study on chronic diseases in the elderly.[10] All inhabitants of a district of the city of Rotterdam aged 55 years or over were invited in random order to the study, and 7983 actually participated (overall response 78%). Because the ophthalmic part became only operational after the study had started, a total of 6780 participants underwent ophthalmic examination. The study was conducted according to the Declaration of Helsinki, and the Medical Ethics Committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline home interviews and examinations were performed from 1990 to mid-1993.

### Retinal vessel measurements

Participants underwent a full eye examination at baseline including simultaneous stereoscopic fundus color photography of the optic disc ($20^0$ field, Topcon Optical Company, Tokyo, Japan) after pharmacological mydriasis. The transparencies from both eyes were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan), and for each participant the digitized image with the best quality (left or right eye) was analyzed with the Retinal Vessel Measurement System (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison).[4] Four trained graders performed these measurements masked for participant characteristics. For each participant one summary value was calculated for the arteriolar diameters of the blood column and one for the venular diameters (in μm) after correction for differences in magnification due to refractive status of the eye; the AVR was defined as the ratio of arteriolar to venular diameters.[11-15]

In a random sub-sample of 100 participants we found no differences between the right and left eyes for the arteriolar and venular diameters.

Pearson's correlation coefficients for inter-grader agreement were for arteriolar diameters 0.67-0.80, for venular diameters 0.91-0.94 and for AVR 0.75-0.84. For intra-grader agreement the corresponding figures were 0.69-0.88, 0.90-0.95 and 0.72-0.90.

**Assessment of stroke**

History of stroke at baseline was assessed and verified as described previously.[3] Once participants entered the Rotterdam Study, they were continuously monitored for incident stroke or transient ischemic attacks through automated linkage of the study database with files from general practitioners and the municipality. Also nursery home physician's files were scrutinized. For reported events, additional information (including brain images) was obtained from hospital records. Research physicians reviewed information on all possible strokes and transient ischemic attacks; an experienced stroke neurologist (P.J.K.) verified all diagnoses masked for retinal vessel measurements. Subarachnoid hemorrhages were excluded. Follow-up was completed until January 1, 2002 for 97.1% of all potential person years.[16]

**Definitions of stroke subtypes**

Cerebral infarction was diagnosed when a patient had typical symptoms and other diagnoses were ruled out on CT or MRI made within four weeks after the stroke occurred. A stroke was also classified as cerebral infarction when indirect evidence (deficit limited to one limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) pointed at an ischemic nature. Primary intra-cerebral hemorrhage was diagnosed when a relevant hemorrhage was shown on CT or MRI scan, or the persons lost consciousness permanently or died within hours after onset of focal signs. If a stroke did not match any of these criteria, it was classified as unspecified.

**Assessment of confounders**

Baseline blood pressure was measured in sitting position at the right brachial artery with a random-zero sphygmomanometer. In the analyses we used the average of two measurements taken at one occasion. Body mass index (BMI) was computed as weight divided by height squared. Non-fasting serum total and HDL cholesterol concentrations were determined by an automated enzymatic procedure.[17] Diabetes mellitus was considered present if participants reported use of antidiabetic medication or when random or post-load serum glucose level was greater than 11 mmol/l. Serum levels of

C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Immage® high sensitive CRP, Beckman Coulter, USA). Atherosclerotic plaques, assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides, were defined as focal thickening of the vessel wall (of at least 1.5 times the average intima-media thickness) relative to adjacent segments with or without calcified components. The carotid artery plaque score (range: 0-6) reflected the number of these locations with plaques.[18] Information on smoking (categorized as current, former or never) and antihypertensive medication use was obtained during the home interview.

**Study sample**

Of the 6780 participants in the ophthalmic part of the Rotterdam Study, 199 were diagnosed with prevalent stroke. Of the remaining 6581 participants at risk of stroke, for 349 we had no fundus transparencies due

**Table 1**. Baseline Characteristics

| | Participants* | Non-participants† | Adjusted differences‡ (95% CI)§ |
|---|---|---|---|
| Number (n) | 5540 | 1041 | |
| Age (years) | 67.8 (8.1) | 75.6 (10.5) | 7.7 (7.2; 8.3)\|\| |
| Gender (%, female) | 59.0 | 63.0 | 0.1 (-3.5; 3.3) |
| Institutionalized (%) | 3.0 | 24.0 | 11.3 (9.8; 12.8)\|\| |
| Smoking (%, current) | 24.0 | 19.0 | 0.9 (-2.0; 3.8) |
| Diabetes Mellitus (%) | 9.0 | 14.0 | 1.2 (-0.9; 3.3) |
| Systolic blood pressure (mmHg) | 138.3 (22.0) | 143.5 (24.1) | 0.3 (-1.2; 1.8) |
| Diastolic blood pressure (mmHg) | 73.7 (11.3) | 73.4 (12.9) | 0.7 (-0.1; 1.5) |
| Number of carotid artery plaques ≥ 4 (%) | 16 | 23 | 0.8 (-2.1; 3.6) |
| Body mass index (kg/m$^2$) | 26.3 (3.7) | 26.0 (3.8) | -0.3 (-0.6; -0.1)\|\| |
| Serum total cholesterol (mmol/l) | 6.64 (1.20) | 6.54 (1.25) | 0.03 (-0.11; 0.05) |
| Serum HDL# cholesterol (mmol/l) | 1.35 (0.36) | 1.34 (0.37) | 0.00 (-0.02; 0.02) |
| C-reactive protein (mg/l) | 3.15 | 4.35 | 0.66 (0.16; 1.16)\|\| |

Presented as unadjusted means (standard deviation) or percentages

* Participants with a gradable fundus transparency on at least one eye
† No (n = 349) or ungradable (n = 692) transparencies
‡ Age and gender adjusted if applicable
§ CI = confidence interval
|| Significant ($p < 0.05$)
# High-density lipoprotein

to logistic reasons and for 692 the fundus transparencies were ungradable. After excluding these persons, a total of 5540 participants remained for the current analyses.

**Statistical analysis**

Analysis of covariance, adjusted for age and gender, was used to compare baseline characteristics of participants with non-participants. Associations of baseline retinal vessel diameters and arteriolar-to-venular ratio with incident stroke and cerebral infarction were analyzed by means of Cox proportional hazards models. Hazard ratios (HR) with corresponding 95% confidence intervals (CI) for stroke and cerebral infarction were calculated by analyzing retinal vessel diameters both linearly (per standard deviation (SD)) and in quartiles. Initial adjustments were made for age and gender; we additionally adjusted for baseline smoking, diabetes mellitus, carotid artery plaque score, body mass index, total and HDL cholesterol, CRP, systolic and diastolic blood pressure, and antihypertensive medication. Participants were followed either until onset of first-ever incident stroke, death or January 1, 2002. All analyses were performed using SPSS (Windows version 11.0; SPSS inc., Chicago, Illinois).

## RESULTS

Comparison of baseline characteristics between participants and non-participants is given in table 1. Adjusted differences showed that those who were excluded were older and more often institutionalized and had a slightly higher CRP and lower BMI. The mean arteriolar diameter was 146.9µm (range: 92.2-235.7; standard deviation (SD): 14.4), venular diameter 222.0µm

**Table 2.** Hazard ratios* of incident stroke and cerebral infarction per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio

| | Stroke (n = 411) | Cerebral infarction (n = 259) |
|---|---|---|
| Arteriolar narrowing† | 1.02 (0.93-1.13) | 1.02 (0.90-1.15) |
| Venular dilatation‡ | 1.12 (1.02-1.24) | 1.15 (1.02-1.29) |
| Arteriolar-to-venular ratio§ | 1.14 (1.04-1.26) | 1.17 (1.03-1.32) |

* Hazard ratios (95% confidence interval) were adjusted for age and gender
† Per standard deviation decrease in arteriolar diameter
‡ Per standard deviation increase in venular diameter
§ Per standard deviation decrease in arteriolar-to-venular ratio

**Table 3.** Hazard ratios* of incident stroke and cerebral infarction per quartile of baseline retinal vessel diameters or arteriolar-to-venular ratio

| Quartiles [range] | Stroke (n = 411) | Cerebral infarction (n = 259) |
|---|---|---|
| Arteriolar diameter (µm) | | |
| 4. [156.4-204.7] | 1.0 (reference) | 1.0 (reference) |
| 3. [147.1-156.4] | 1.11 (0.84-1.47) | 1.09 (0.78-1.54) |
| 2. [137.8-147.1] | 1.10 (0.83-1.46) | 1.01 (0.71-1.44) |
| 1. [95.0-137.8] | 1.05 (0.79-1.39) | 0.96 (0.68-1.37) |
| Q1 versus Q2-4† | 0.98 (0.78-1.22) | 0.93 (0.70-1.23) |
| Venular diameter (µm) | | |
| 1. [135.1-209.0] | 1.0 (reference) | 1.0 (reference) |
| 2. [209.0-222.1] | 1.03 (0.78-1.36) | 1.04 (0.73-1.48) |
| 3. [222.1-235.4] | 1.08 (0.82-1.43) | 1.04 (0.73-1.49) |
| 4. [235.4-313.6] | 1.33 (1.01-1.74) | 1.39 (0.99-1.95) |
| Q4 versus Q1-3‡ | 1.28 (1.03-1.60) | 1.34 (1.03-1.77) |
| Arteriolar-to-venular ratio | | |
| 4. [0.70-0.87] | 1.0 (reference) | 1.0 (reference) |
| 3. [0.66-0.70] | 1.31 (0.99-1.73) | 1.33 (0.93-1.90) |
| 2. [0.63-0.66] | 0.97 (0.71-1.31) | 0.97 (0.66-1.43) |
| 1. [0.49-0.63] | 1.65 (1.26-2.15) | 1.73 (1.23-2.43) |
| Q1 versus Q2-4§ | 1.52 (1.23-1.86) | 1.57 (1.22-2.04) |

* Hazard ratios (95% confidence interval) were adjusted for age and gender
† Lowest versus all other quartiles of retinal arteriolar diameters
‡ Highest versus all other quartiles of retinal venular diameters
§ Lowest versus all other quartiles of retinal arteriolar-to-venular ratio

(range: 135.1-313.6; SD: 20.9) and AVR 0.66 (0.48-1.02; SD: 0.06). During a total of 47,103 person-years (mean follow-up per person: 8.5 years (SD: 2.6)) 411 participants had incident stroke, including 259 cerebral infarctions.

Table 2 shows that per SD increase in venular diameters the risk of incident stroke increased significantly by 12%. Smaller arteriolar diameters were not associated with an increased risk of stroke. The resulting arteriolar-to-venular ratio revealed that each SD decrease was associated with a 14%

increased risk of stroke. The relationship between retinal vessel diameters and cerebral infarction is presented in table 2. Larger venular diameters were also associated with the risk of cerebral infarction (HR per SD increase: 1.15; 95% CI: 1.02-1.29).

Table 3 presents the analyses after categorizing retinal vessel diameters into quartiles. Participants in the highest quartile of venular diameters had a 33% increased risk of stroke compared with those in the lowest quartile. Therefore, we grouped the three lowest quartiles of venular diameters. Compared with participants in the lowest three quartiles those in the highest quartile had a significantly increased risk of stroke of 28%. Quartiles of arteriolar diameters were not related to the risk of stroke. Participants in the lowest quartile of AVR had 1.65 times higher risk of stroke compared with those in the highest quartile. Comparing the lowest quartile of AVR to all the others resulted in a 1.52 times increased risk of stroke. In table 3 the associations between quartiles of retinal vessel diameters and cerebral infarction are presented.

Finally, in table 4 the HRs for the association between venular diameters and stroke are presented after additional adjustment for other cardiovascular risk factors. The strength of the association hardly changed with additional adjustment and remained significant. After adjusting for all cardiovascular risk factors, each SD increase in venular diameter resulted in a 13% increased risk of stroke (table 4). For cerebral infarction the corresponding risk increased

**Table 4.** Hazard ratios* of stroke and cerebral infarction per standard deviation increase in retinal venular diameters additionally adjusted for other cardiovascular risk factors

| | **Stroke (n = 411)** | **Cerebral infarction (n = 259)** |
|---|---|---|
| Age and gender | 1.12 (1.02-1.24) | 1.15 (1.02-1.29) |
| Age, gender and carotid artery plaque score | 1.13 (1.02-1.26) | 1.16 (1.02-1.32) |
| Age, gender, total and HDL† cholesterol | 1.12 (1.01-1.23) | s1.14 (1.01-1.29) |
| Age, gender and body mass index | 1.12 (1.02-1.24) | 1.14 (1.01-1.29) |
| Age, gender and C-reactive protein | 1.12 (1.01-1.24) | 1.15 (1.01-1.31) |
| Age, gender and smoking | 1.11 (1.00-1.22) | 1.12 (0.99-1.27) |
| Age, gender and diabetes mellitus | 1.12 (1.02-1.23) | 1.14 (1.01-1.29) |
| Age, gender, blood pressure and medication‡ | 1.13 (1.03-1.25) | 1.16 (1.02-1.31) |
| Fully adjusted | 1.13 (1.01-1.27) | 1.15 (1.00-1.33) |

* Hazard ratios (with corresponding 95% confidence interval)

† High-density lipoprotein

‡ Systolic and diastolic blood pressure and anti-hypertensive medication

by 15%. In the fully adjusted models, smaller arteriolar diameters were neither related to the risk of stroke (HR per SD decrease: 0.97; 95% CI: 0.87-1.09), nor to the risk of cerebral infarction (HR per SD decrease: 0.98; 95% CI: 0.84-1.13). The association of AVR with stroke (HR per SD decrease: 1.08; 95% CI: 0.97-1.21) and cerebral infarction (HR per SD decrease: 1.11; 95% CI: 0.96-1.29) attenuated after additional adjustments.

## DISCUSSION

We found that larger retinal venular diameters were associated with an increased risk of stroke or cerebral infarction, its major subtype, independently of other cardiovascular risk factors.

The strengths of this study are its population-based design, the intense stroke case finding, the nearly complete follow-up, the long period of follow-up, and the large number of cases. Another advantage is the standardized and detailed quantification of retinal vessel diameters on fundus transparencies. Furthermore, we used the improved Parr-Hubbard formulas and Littmann's correction to approximate absolute intra-luminal diameters.[12,15]

Some possible limitations warrant consideration. Non-participants were on average older and more often lived in nursing homes. However, since the two groups hardly differed in any of the other cardiovascular risk factors we do not think this has introduced selection bias. In some cases stroke may have been misclassified. Since strokes were classified without knowledge of retinal vessel measurements, misclassification, if any, will have been non-differential leading to an underestimation of the risks. Finally, we could not examine the relationship between retinal vessel diameters and primary intra-cerebral hemorrhage due to small number of cases, and for cerebral infarction we were not able to specify the ischemic subtypes in all cases.

Retinal venular diameters might be related to stroke through several possible mechanisms. We have previously shown that larger venular diameters were related to cardiovascular risk factors such as atherosclerosis as measured by more plaques in the carotid arteries, higher levels of total cholesterol, lower levels of HDL, higher levels of inflammatory markers (such as erythrocyte sedimentation rate and leukocyte count) and smoking.[9] Because these factors are also related to the risk of stroke,[2,3,19,20] these factors may explain the observed association. After adjustment for all these risk factors simultaneously the relationship between venular dilatation and stroke persisted, suggesting that still other mechanisms should be considered.

Increase in venular diameters may result from retinal hypoxia.[21]

Venular and to a lesser extent arteriolar dilatation has been described not only in the early stages of diabetic retinopathy, but also in venous stasis retinopathy, which is commonly seen in patients with occlusive disease of the carotid artery.[22] Cerebral and retinal hypoperfusion seems to be an important factor in the development of venous stasis retinopathy. Treatment with carotid endarterectomy or retinal photocoagulation leads to reduction of venular diameters, probably due to less retinal hypoxia.[22,23] Therefore, a possible explanation is that venular dilatation might be a general feature reflecting a decreased blood flow and diffuse cerebral ischemia, making the brain tissue more prone to infarction.

Smaller arteriolar diameters were related to higher blood pressures.[9] It has been suggested that arteriolar narrowing reflected vasoconstriction, intimal thickening and medial hyperplasia.[9] Based on these observations, we hypothesized that smaller arteriolar diameters would be related to risk of stroke. However, our study could not confirm this hypothesis. The lack of an association could be due to the fact that in conditions reflecting retinal or cerebral hypoxia, the arterioles still possess, despite increased arterial stiffness, the ability to dilate, though less pronounced than in the venules.[9,21,22] These opposing effects, arteriolar narrowing and widening, might blur the relationship between arteriolar diameters and stroke.

Most studies that examined retinal vascular signs in relationship to cerebral pathology were hospital-based and conducted in patients with rare hereditary diseases involving small vessels like cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) or hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS).[24-26] More recently, it was shown that persons with cerebral white matter lesions and lacunes more often had retinal changes such as generalized arteriolar narrowing and arterio-venous nicking.[26] In another study with an average follow-up of 3.5 years a lower AVR was associated with an increased risk of stroke (HR 1$^{st}$ versus 5$^{th}$ quintile: 1.98 (1.09-3.60)).[7] The authors did not present arteriolar and venular diameters separately. In line with these findings, our study confirmed the association between the AVR and stroke. More importantly, our data revealed that venular dilatation rather than arteriolar narrowing explained this association.

In conclusion, we observed that retinal venular diameters were related to an increased risk of stroke or its major subtype cerebral infarction. These associations were independent of other cardiovascular risk factors. The mechanisms underlying venular dilatation may provide intriguing new directions for research into the pathogenesis of cerebrovascular diseases.

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# Chapter 4.2

# Retinal Vessel Diameters and Cerebral Small Vessel Disease: The Rotterdam Scan Study


## SUMMARY

*The direct visualization of retinal vessels provides a unique opportunity to study cerebral small vessel disease, because these vessels share many features. It was reported that persons with smaller retinal arteriolar-to-venular ratio tended to have more white matter lesions on MRI. It is unclear if this is due to arteriolar narrowing or venular dilatation. We investigated if smaller arteriolar or larger venular diameters were related to severity and progression of cerebral small vessel disease.*

*We studied 490 randomly selected elderly without dementia (60-90 years) from a population-based cohort study. At baseline (1990-1993), retinal arteriolar and venular diameters were measured on digitized images of one eye of each participant. In 1995-1996, participants underwent cerebral MRI scanning. We rated severity of periventricular white matter lesions on a 9-point scale, approximated a total subcortical white matter lesion volume (range: 0-29.5 ml), and rated the presence of lacunar infarcts. On average 3.3 years later, 279 persons had a second MRI. We rated changes in periventricular and subcortical white matter lesions with a semi-quantitative scale, and classified progression as no, minor and marked. An incident infarct was a new infarct on the follow-up MRI.*

*Neither larger venular, nor smaller arteriolar diameters were related to the severity of cerebral small vessel disease. Larger venular diameters were, however, associated with marked white matter lesion progression and incident lacunar infarcts. Odds ratios (OR) per standard deviation increase were 1.71 (95% confidence interval [CI]: 1.11-2.61) for periventricular, 1.72 (95% CI: 1.09-2.71) for subcortical white matter lesion progression, and 1.59 (95% CI: 1.06-2.39) for incident lacunar infarcts. These associations were independent of other cardiovascular risk factors, except for the association with incident lacunar infarcts, which attenuated (OR: 1.24; 95% CI: 0.72-2.12). No association was observed between arteriolar diameters and progression of cerebral small vessel disease.*

*In conclusion, retinal venular dilatation was related to progression of cerebral small vessel disease. The mechanisms underlying venular dilatation deserve more attention, as they may provide exciting new clues into the pathophysiology of cerebral small vessel disease.*


Submitted

Cerebral magnetic resonance imaging (MRI) in elderly people frequently reveals white matter lesions and lacunar infarcts. Prevalence ranges from 5% to 90% in different studies (van Dijk *et al.*, 2002). These lesions are related to incident stroke and may contribute to the development and dementia (Barber *et al.*, 1999; Kobayashi *et al.*, 1997; Prins *et al.*, 2004a; Vermeer *et al.*, 2003b). They reflect ischemic small vessel disease, though the exact patho-physiological mechanism is unknown (Pantoni and Garcia, 1997). Several studies point towards increasing age, hypertension and markers of atherosclerosis as main risk factors (Bots *et al.*, 1993; Liao *et al.*, 1997; Longstreth *et al.*, 1996; Schmidt *et al.*, 1997). The pathological status of cerebral small vessels is difficult to assess in vivo. Most non-invasive markers of vascular pathology are related to blood vessels outside the brain and may not represent local cerebral abnormalities. The retinal vessels provide unique opportunities to study cerebral small vessel disease, because they share similar anatomy, physiology, and embryology (Wong *et al.*, 2001).

Recently, a semi-automated system was developed to measure retinal vessel diameters. Subsequently, a smaller arteriolar-to-venular ratio was suggested to reflect generalized arteriolar narrowing and was associated with cardiovascular diseases (Hubbard *et al.*, 1999; Wong *et al.*, 2002b). In

the Atherosclerosis Risk in Communities Study, persons with a smaller arteriolar-to-venular ratio tended to have more white matter lesions (Wong *et al.*, 2002a). However, it can be questioned whether the arteriolar-to-venular ratio reflects solely retinal arteriolar narrowing, because venular width does not remain constant in various pathological conditions. We reported that smaller arteriolar diameters were related to higher blood pressures, whereas larger venular diameters were related to markers of atherosclerosis and inflammation (Ikram *et al.*, 2004).

We investigated whether smaller arteriolar or larger venular diameters in the retina were related to the severity and progression of cerebral small vessel disease.

## PARTICIPANTS AND METHODS

### Study population

The Rotterdam Scan Study is a population-based cohort study designed to investigate the etiology and natural history of age-related brain changes in the elderly (de Leeuw *et al.*, 2001). In 1995-1996, we randomly selected participants, aged 60 to 90 years, stratified by gender and 5-year age strata from two on-going population-based cohort studies, the Rotterdam Study (Hofman *et al.*, 1991) and the Zoetermeer Study (Hofman *et al.*, 1983). Persons who were demented at baseline were excluded. Complete information, including a cerebral MRI scan, was obtained in 1,077 individuals, who gave written informed consent (response rate 63%). The study was conducted according to the tenets of the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol.

Of these 1077 participants, 563 originated from the Rotterdam Study. During the baseline phase, which took place between 1990-1993, they had an eye examination, which included taking fundus color transparencies. To obtain the study sample for the current analyses, 72 persons without transparencies due to logistic reasons and one person with ungradable transparencies were excluded, leaving 490 persons in whom retinal vessel diameters could be measured. In 1999-2000, 279 of the 435 participants who were alive and without contraindications underwent a second MRI scan.

### Retinal vessel measurements

The ophthalmic examination at baseline included taking simultaneous stereoscopic fundus transparencies centered on the optic disc of both eyes with a telecentric fundus camera (pharmacological mydriasis, $20^0$

field, Topcon Optical Company, Tokyo, Japan) (Ikram *et al.*, 2004). These transparencies were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan) and for each participant one eye with the best image quality was analyzed with a semi-automated system (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison). Per eye one summary value was calculated for the diameters of the blood column in the retinal arterioles and one for the venules (in μm) after correction for differences in magnification due to refractive status of the eye. The arteriolar-to-venular ratio was defined as the ratio of arteriolar to venular diameters (Knudtson *et al.*, 2003; Littmann, 1988).

In a random sub-sample of 100 participants we found no statistically significant differences between right and left eyes for the arteriolar and venular diameters. Four trained graders performed all measurements masked for participant characteristics. Pearson's correlation coefficients for inter-grader agreement were for arteriolar diameters 0.67-0.80, for venular diameters 0.91-0.94 and for arteriolar-to-venular ratio 0.75-0.84. For intra-grader agreement the corresponding figures were 0.69-0.88, 0.90-0.95 and 0.72-0.90.

**MRI scanning**

In 1995-1996, axial T1-, T2- and proton density (PD)-weighted cerebral MR scans were made on a 1.5-Tesla MRI scanner (MR VISION, Siemens) (de Leeuw et al., 2001). In 1999-2000, participants underwent a second MRI on the same MR VISION scanner with the same sequences.

**White matter lesions**

White matter lesions were considered present when visible as hyper-intense on proton density and T2-weighted images, without prominent hypo-intensity on T1-weighted scans. We considered white matter lesions to be in the periventricular region if they were directly adjacent to the ventricle; otherwise we called them subcortical. We scored periventricular white matter lesions semiquantatively in three regions (lesions adjacent to the frontal horns, the lateral walls, and the occipital horns of the lateral ventricle) resulting in a total score ranging from 0-9. For subcortical white matter lesions we approximated a total volume based on number and size of lesions (volume range: 0-29.5 ml). Both intra-and inter-rater studies (n=100) showed a good to excellent agreement ($\varkappa$ = 0.79-0.90, r = 0.88-0.95) (de Leeuw *et al.*, 2001).

We used a new scale to measure change in white matter lesions severity, since commonly used rating scales designed for cross-sectional assessments of white matter lesions are not well suited for measuring

change (Prins *et al.*, 2004b). Two raters independently assessed progression of white matter lesions severity on digital T2- and PD-weighted images by direct scan comparison. Raters were masked to all clinical information. They scored differences in white matter lesion severity in the three periventricular regions of both hemispheres (periventricular score range –6 to +6) and in the subcortical white matter of the four lobes of both hemispheres (subcortical score range: -8 to +8). The rating showed good interobserver (interclass correlation coefficient 0.75-0.93) and intraobserver agreement (intraclass correlation coefficient 0.70-0.93). If raters disagreed one point or less on the scale, the mean of the ratings was used; if more, a consensus meeting was held. Progression was defined as an increase of one point or more between baseline and follow-up. We categorized progression into no (score < 1), minor (score 1-2.5), and marked progression (score 3 or higher).

**Lacunar infarcts**

The presence of brain infarcts was rated similarly at the baseline and second MRI (Vermeer *et al.*, 2003a). We defined brain infarcts as areas of focal hyperintensity on T2-weighted images sized ≥ 3 mm. Areas of hyperintensity in the white matter also had to have corresponding prominent hypointensity on T1-weighted images, in order to distinguish them from white matter lesions. Lacunar infarcts were defined as infarcts 3 to 20 mm in size and located in the subcortical white matter or basal ganglia. Non-lacunar infarcts were excluded from the analyses of lacunar infarcts. A new infarct on the follow-up MRI was classified as incident lacunar infarct.

**Cardiovascular risk factors**

Baseline blood pressure was measured in sitting position at the right brachial artery with a random-zero sphygmomanometer. In the analyses we used the average of two measurements taken at one occasion. Body mass index (BMI) was computed as weight divided by height squared. Non-fasting serum total and HDL cholesterol levels were determined by an automated enzymatic procedure (van Gent *et al.*, 1977). Diabetes mellitus was considered present if participants reported use of antidiabetic medication or when random or post-load serum glucose level was greater than 11 mmol/l. The presence of atherosclerotic plaques was assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides, resulting in a carotid artery plaque score ranging from 0 to 6 (Bots *et al.*, 1996). Serum levels of C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Immage® high sensitive CRP, Beckman

Coulter, USA). Information on smoking (categorized as current, former or never) was obtained during the home interview.

**Statistical analyses**

We used age and gender adjusted analysis of covariance to analyze whether baseline risk factors differed between people with and without a second MRI assessment. With linear regression analyses we assessed the relationship between baseline retinal vessel diameters and severity of periventricular and subcortical white matter lesions and with multinomial logistic regression analyses their relationship with white matter lesion progression. We used logistic regression analyses to study the association between retinal vessel diameters and prevalent as well as incident lacunar infarcts. All analyses were performed adjusted for age and gender, and additionally for other cardiovascular risk factors with SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

Baseline characteristics of participants with and without repeated MRI assessments are presented in table 1. Non-participants at follow-up were on average older than those who did have a repeated MRI assessment. With respect to all other cardiovascular risk factors, there were no significant differences between the two groups after adjusting for age and gender.

The mean follow-up period between the first and second MRI was 3.3 years (standard deviation (SD): 0.2 years). During this period, 82 participants showed white matter progression in the periventricular region, of whom 31 had marked progression. A total of 89 participants showed progression in the subcortical region, of whom 25 had marked progression. There were 27 participants who had a new lacunar infarct on the follow-up MRI.

Retinal vessel diameters were neither associated with the severity of white matter lesions, nor with prevalent lacunar infarcts (table 2).

Larger retinal venular diameters were associated with marked progression of both periventricular and subcortical white matter lesions and with incident lacunar infarcts (table 3). Smaller arteriolar diameters were neither related to white matter lesion progression, nor to incident lacunar infarcts.

Finally, table 4 presents the relation between retinal venular diameters and cerebral small vessel disease after additional adjustment for other cardiovascular risk factors. Each SD increase in venular diameters resulted

**Table 1**. Baseline characteristics

| | All participants | Participants with repeated MRI assessment | Non-participants at follow-up | Adjusted differences* (95% CI)† |
|---|---|---|---|---|
| Number (n) | 490 | 279 | 211 | |
| Age (years) | 68.4 (7.8) | 67.0 (7.6) | 70.3 (7.8) | 3.3 (1.9; 4.7)‡ |
| Gender (% female) | 49 | 49 | 49 | 0.8 (-8.4; 9.9) |
| Diabetes Mellitus (%) | 6.3 | 4.8 | 8.3 | 2.3 (-2.5; 7.0) |
| Smoking (% current) | 22 | 23 | 20 | 0.0 (-7.5; 7.6) |
| Systolic blood pressure (mmHg) | 136.7 (19.9) | 134.2 (19.3) | 139.9 (20.2) | 3.5 (-0.03; 6.9) |
| Diastolic blood pressure (mmHg) | 73.1 (10.8) | 72.6 (10.6) | 73.7 (11.2) | 1.5 (-0.4; 3.5) |
| Body mass index (kg/m$^2$) | 26.3 (3.3) | 26.1 (3.2) | 26.5 (3.3) | 0.3 (-0.3; 0.9) |
| Carotid artery plaque score ≥ 4 (%) | 17 | 15 | 21 | 2.0 (-5.0; 8.9) |
| C-reactive protein (mg/l) | 2.85 | 2.51 | 3.31 | 0.57 (-0.30; 1.44) |
| Serum total cholesterol (mmol/l) | 6.65 (1.3) | 6.71 (1.3) | 6.58 (1.2) | -0.10 (-0.32; 0.12) |
| Serum HDL§ cholesterol (mmol/l) | 1.33 (0.4) | 1.33 (0.3) | 1.33 (0.5) | 0.00 (-0.07; 0.07) |
| Periventricular WML\|\| (range: 0-9) | 2.68 | 2.46 | 2.97 | 0.08 (-0.28; 0.43) |
| Subcortical WML\|\| (ml) | 1.81 | 1.59 | 2.10 | -0.01 (-0.60; 0.57) |
| All infarcts (%) | 26 | 23 | 30 | 1.1 (-6.7; 8.8) |
| Lacunar infarcts# (%) | 22 | 20 | 25 | 0.0 (-7.0; 7.9) |
| Retinal arteriolar diameter (µm) | 147.8 (14.2) | 147.8 (13.9) | 147.9 (14.6) | 0.3 (-2.4; 2.9) |
| Retinal venular diameter (µm) | 223.8 (20.6) | 223.3 (20.1) | 224.5 (21.2) | 3.0 (-0.7; 6.9) |
| Retinal arteriolar-to-venular ratio | 0.66 (0.06) | 0.66 (0.06) | 0.66 (0.06) | -0.008 (-0.018; 0.003) |

Presented as unadjusted means (standard deviation) or percentages

* Age and gender adjusted if applicable
† CI = confidence interval
‡ Significant ($p < 0.05$) compared to those with repeated MRI assessment
§ HDL = high-density lipoprotein
|| WML = white matter lesions
# Non-lacunar infarcts excluded

**Table 2**. Cross-sectional association between retinal vessel diameters and severity of cerebral small vessel disease (n = 490)

| | Periventricular WML* (grade) | Subcortical WML* (ml) | Lacunar infarct (OR, (95% CI))† |
|---|---|---|---|
| Arteriolar narrowing‡ | 0.002 (-0.17; 0.17) | 0.01 (-0.21; 0.36) | 1.14 (0.91-1.44) |
| Venular dilatation§ | -0.004 (-0.18; 0.17) | 0.08 (-0.21; 0.37) | 1.07 (0.85-1.35) |
| Arteriolar-to-venular ratio\|\| | 0.04 (-0.14; 0.22) | 0.18 (-0.11; 0.47) | 1.24 (0.98-1.58) |

All models adjusted for age and gender

* WML = white matter lesions
† OR = odds ratio; CI = confidence interval
‡ Per standard deviation decrease in arteriolar diameter
§ Per standard deviation increase in venular diameter
|| Per standard deviation decrease in arteriolar-to-venular ratio

in a 1.8 times increased risk of marked periventricular white matter lesion progression, and in a 2.8 times increased risk of marked subcortical white matter progression. Persons with larger venular diameters tended to have more incident lacunar infarcts, however this association attenuated after additional adjustments.

## DISCUSSION

We found that especially larger retinal venular diameters were associated with marked progression of periventricular and subcortical white matter lesions independent of other cardiovascular risk factors. Also, persons with larger venular diameters tended to have more incident lacunar infarcts.

Some methodological issues warrant consideration. Those who had the first MRI were significantly younger and had lower blood pressures compared to non-responders. Because old age and elevated blood pressure are known risk factors for both white matter lesions and lacunar infarcts, persons with more severe brain lesions could have been underrepresented in the cross-sectional analyses. This bias might have resulted in a lack of any association in the cross-sectional analyses. Participation at follow-up was also associated with age. However, there were no significant differences in other cardiovascular risk factors between those with and without a second MRI suggesting that selection bias played a limited role in the longitudinal analyses (table 1).

Photographs were not taken synchronized on the cardiac cycle, leading

**Table 3.** Odds ratios* of white matter lesion progression and incident lacunar infarct per standard deviation difference in baseline retinal vessel measurements (n = 279)

| | Periventricular WML† progression | | | Subcortical WML† progression | | | Incident lacunar infarct |
|---|---|---|---|---|---|---|---|
| | Any (n = 82) | Minor (n = 51) | Marked (n = 31) | Any (n = 89) | Minor (n = 64) | Marked (n = 25) | All (n = 27) |
| Arteriolar narrowing‡ | 0.93 (0.70-1.22) | 1.10 (0.78- 1.56) | 0.77 (0.52-1.14) | 0.85 (0.65-1.11) | 0.92 (0.67-1.25) | 0.76 (0.49-1.16) | 0.81 (0.54-1.22) |
| Venular dilatation§ | 1.33 (0.99-1.78) | 1.12 (0.79-1.60) | 1.71 (1.11-2.61) | 1.22 (0.93-1.61) | 1.06 (0.78-1.45) | 1.72 (1.09-2.71) | 1.59 (1.06-2.39) |
| Arteriolar-to-venular ratio\|\| | 1.22 (0.92-1.61) | 1.20 (0.85-1.67) | 1.25 (0.84-1.92) | 1.03 (0.79-1.33) | 0.95 (0.71-1.28) | 1.28 (0.83-2.00) | 1.29 (0.84-1.98) |

* Age and gender adjusted odds ratios with corresponding 95% confidence intervals

† WML = white matter lesions

‡ OR per standard deviation decrease in retinal arteriolar diameters

§ OR per SD increase in retinal venular diameters

|| OR per SD decrease in arteriolar-to-venular ratio

**Table 4**. Odds ratios* of white matter lesion progressions and incident lacunar infarcts per standard deviation increase in retinal venular diameters additionally adjusted for other cardiovascular risk factors (n = 279)

| | Periventricular WML† progression | | Subcortical WML† progression | | Incident lacunar Infarcts |
|---|---|---|---|---|---|
| | Minor | Marked | Minor | Marked | |
| Age and gender | 1.12 (0.79-1.60) | 1.71 (1.11-2.61) | 1.06 (0.78-1.45) | 1.72 (1.09-2.71) | 1.59 (1.06-2.39) |
| Age, gender and cholesterol‡ | 1.10 (0.77-1.57) | 1.80 (1.17-2.78) | 1.05 (0.76-1.44) | 1.80 (1.12-2.89) | 1.59 (1.06-2.39) |
| Age, gender and body mass index | 1.14 (0.80-1.63) | 1.63 (1.07-2.52) | 1.05 (0.77-1.44) | 1.68 (1.06-2.66) | 1.58 (1.05-2.39) |
| Age, gender and carotid artery plaques | 1.16 (0.80-1.69) | 1.65 (1.06-2.57) | 1.11 (0.80-1.54) | 1.95 (1.21-3.17) | 1.43 (0.92-2.22) |
| Age, gender and C-reactive protein | 1.07 (0.75-1.53) | 1.60 (1.03-2.47) | 1.03 (0.75-1.41) | 1.58 (1.00-2.52) | 1.52 (1.00-2.30) |
| Age, gender and smoking | 1.08 (0.74-1.57) | 1.64 (1.05-2.57) | 1.06 (0.77-1.46) | 1.69 (1.07-2.68) | 1.67 (1.10-2.54) |
| Age, gender and diabetes mellitus | 1.25 (0.85-1.84) | 1.79 (1.13-2.83) | 1.06 (0.75-1.51) | 1.77 (1.10-2.85) | 1.40 (0.90-2.17) |
| Age, gender and blood pressure§ | 1.12 (0.78-1.61) | 1.73 (1.12-2.68) | 1.07 (0.78-1.47) | 1.83 (1.13-2.97) | 1.62 (1.07-2.45) |
| Fully adjusted | 1.17 (0.74-1.85) | 1.76 (1.01-3.00) | 1.09 (0.74-1.60) | 2.75 (1.36-5.59) | 1.24 (0.72-2.12) |

* Odds ratios with corresponding 95% confidence intervals

† WML = white matter lesions

‡ Total and high-density lipoprotein cholesterol

§ Systolic and diastolic blood pressure

to variation in retinal vessel diameter due to pulsatility. Because retinal vessel measurements, covariates and brain lesions were assessed independently from each other, misclassification, if any, will have been random and would have resulted in an underestimation of the associations.

Previously, we showed that larger venular diameters were related to atherosclerosis (as measured by more plaques in the carotid arteries), higher levels of total cholesterol, lower levels of HDL, higher levels of inflammatory markers (such as erythrocyte sedimentation rate and leukocyte count) and smoking (Ikram *et al.*, 2004). Because these risk factors are also related to the development of white matter lesions and lacunar infarcts, they may explain the observed associations (Bots *et al.*, 1993; Breteler *et al.*, 1994). However, adjustment for these risk factors hardly changed the association between venular dilatation and cerebral small vessel disease suggesting that other mechanisms are involved.

It has been hypothesized that retinal venular dilatation occurs in response to retinal hypoxia. Venular dilatation has been described as one of the earliest changes not only in diabetic retinopathy, but also in the less well-known venous stasis retinopathy, both of which are characterized by retinal hypoxia (Klijn *et al.*, 2002; Zatz and Brenner, 1986). Furthermore, treatment with photocoagulation decreases the oxygen requirement of retinal tissue eventually leading to disappearance of venular dilatation (Carter, 1985; Neupert *et al.*, 1976). Based on these observations, a possible explanation might be that venular dilatation is a general marker of decreased blood flow leading to diffuse cerebral ischemia and increased susceptibility of brain tissue to the development of white matter lesions and lacunar infarcts.

Another explanation might be that retinal venular dilatation in some way reflects cerebral venular abnormalities. In one study, brains with severe white matter lesions showed wall thickening and obstruction of the periventricular veins, a condition known as periventricular venous collagenosis (Moody *et al.*, 1995; Moody *et al.*, 1997). It was suggested that these alterations resulted in an increased venous pressure, venular dilatation, and venular blood-brain barrier disruption. This venous insufficiency could result in ischemic stress from impaired clearance of cellular metabolites in the deep white matter eventually leading to white matter lesions (Moody *et al.*, 1995; Sahlas *et al.*, 2002). It is unkown to what extent such cerebral venular changes might be reflected in the retinal venules.

With regard to arterioles, we previously suggested that arteriolar narrowing reflects vasoconstriction, intimal thickening and medial hyperplasia (Ikram *et al.*, 2004). Hence, we hypothesized that smaller arteriolar diameters

would be related to cerebral small vessel disease. However, our study did not confirm this hypothesis. This could be due to the fact that, despite increased arterial stiffness, arterioles still possess the ability to dilate even in retinal or cerebral hypoxia, though less so than the venules (Ikram *et al.*, 2004; Klein *et al.*, 2004; Klijn *et al.*, 2002). These opposing effects in the same arterioles, narrowing and widening, might result in no apparent association.

In conclusion, we found retinal venular dilatation to be associated with progression of cerebral small vessel disease. In case, future research would focus more on the mechanisms underlying venular abnormalities including dilatation, this might provide exciting new clues into the pathophysiology of cerebral small vessel disease.

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# Chapter 4.3

# Retinal Arteriolar and Venular Diameters and Risk of Dementia: The Rotterdam Study


## ABSTRACT

***Context*** *– Evidence suggests that vascular pathology is not only implicated in vascular dementia but also in Alzheimer's disease. Retinal vessels emerge from the cerebral circulation and the retinal arteriolar-to-venular diameter ratio has been related to cardio- and cerebrovascular diseases. The relationship with dementia, and its major subtypes Alzheimer's disease and vascular dementia has not been determined.*

***Objective*** *– To determine whether retinal arteriolar and venular diameters are associated with incidence of dementia, Alzheimer's disease and vascular dementia.*

***Design and setting*** *– The Rotterdam Study, a population-based, prospective cohort study conducted in the Netherlands.*

***Participants*** *– A total of 5553 participants (55 years or over), who were free of dementia at baseline (1990-1993) and had digitized retinal images.*

***Main outcome measures*** *– Incidence of dementia, Alzheimer's disease and vascular dementia, based on established criteria*

***Results*** *– After a mean follow-up of 6.0 years, 233 participants developed dementia. Alzheimer's disease was diagnosed in 167 and vascular dementia in 38 participants. Larger venular diameters increased the risk of dementia (hazard ratio [HR] per SD increase in venular diameter: 1.21 [95% confidence interval (CI), 1.07; 1.37]). For Alzheimer's disease and vascular dementia results were similar (HRs 1.22 [95% CI 1.06; 1.41] and 1.21 [95% CI 0.89; 1.64] respectively) although non-significant for vascular dementia. Arteriolar diameters were neither associated with dementia nor its major subtypes. After additional adjustment for other cardiovascular risk factors, the results did not alter.*

***Conclusion*** *– Larger retinal venular diameters are associated with an increased risk of dementia and its major subtypes Alzheimer's disease and vascular dementia independently of other vascular risk factors.*


Submitted

Evidence suggests that vascular pathology plays an important role not only in vascular dementia but also in the clinical course and progression of Alzheimer's disease.[1-3] Disruption of the blood-brain barrier, endothelial dysfunction and amyloid deposition in cerebral vessels are common in advanced cases.[4,5] Whether cerebrovascular changes are involved in the pathogenesis of Alzheimer's disease or reflect effects of aging or atrophy secondary to neuronal loss remains unclear.[5] The cerebral circulation is however difficult to access and most non-invasive indicators of vascular pathology relate to vessel beds outside the cerebrum. Retinal vessels provide a useful way to more directly study vascular damage to the brain, because embryological, anatomical and physiological characteristics are similar to the cerebral circulation and the retina is easy to visualize non-invasively.[6,7]

Recently, a semi-automated system has been developed to reliably quantify retinal arteriolar and venular diameters.[8] A lower arteriolar-to-venular ratio (AVR) is commonly used to indicate generalized arteriolar narrowing and has been found to predict cardiovascular disease and stroke.[9-11] The AVR was not related to cognitive impairment in middle-aged persons.[12] However, equating a lower AVR to arteriolar narrowing may be problematic, as larger venular diameters have been associated with higher levels of inflammation

markers and cholesterol, and more sub-clinical atherosclerosis.[13] Given that retinal arterioles and venules respond differently to various pathological conditions, their diameters should also be investigated separately. With respect to dementia these data are currently not available.

We therefore studied the association between retinal arteriolar and venular diameters and the risk of dementia.

## METHODS

### The Rotterdam Study

The Rotterdam Study is a population-based, prospective cohort study consisting of 7,983 participants aged 55 years and over.[14] A smaller number (n = 6780) participated in the ophthalmic part of the study, since eye examinations became operational a few months after the baseline examinations had started. Fundus transparancies were available in 6436 participants and of these, 6432 participants were screened for dementia of whom 213 were diagnosed to be demented at baseline. The cohort at risk of dementia thus comprised of 6219 participants who gave written informed consent. The study was conducted according to the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol.

### Dementia diagnoses

Participants were screened for dementia with a 3-step procedure, which was similar at baseline and follow-up examinations.[15] First, participants were cognitively screened with the Mini-Mental State Examination (MMSE) and the Geriatric Mental State schedule (GMS) organic level.[16,17] Second, if participants scored below 26 on the MMSE or above 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX) was administered, including an informant interview.[18] Finally, participants suspected of having dementia were further examined by a neurologist, a neuropsychologist and, if possible, had magnetic resonance imaging of the brain. In addition, continuous monitoring of the cohort for incident dementia cases took place through computerized linkage between the study database and computerized medical records from general practitioners and through surveillance of Regional Institute for Outpatient Mental Health Care reports. Dementia diagnosis was based on DSM-III-R criteria and diagnoses of the two major subtypes Alzheimer's disease and vascular dementia were subsequently based on the NINCDS-ADRDA and the NINDS-AIREN criteria respectively.[19-21] Diagnoses were made on all existing

information by an expert panel including the neurologist, neuropsychologist and research physician. Follow-up for dementia was virtually complete until december 1999 (99.9%).

**Grading of retinal vessel diameters**

At the baseline ophthalmic examination, simultaneous stereoscopic fundus color transparencies were taken centered on the optic disk ($20^0$ field, Topcon Optical Company, Tokyo, Japan) after pharmacological mydriasis.[22] Transparencies from both eyes were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan). For each subject the digitized image with the best quality was analyzed with the Retinal Vessel Measurement System (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison, USA).[8] Four trained graders performed the assessments, blinded to the clinical characteristics of the participants.

The rationale and procedures to measure and summarize retinal vessel diameters have been described.[8] For current analyses, the revised and improved Parr-Hubbard formulas were used to compute the summary measures for arteriolar and venular diameters.[23] In addition, Littmann's formula was used to correct for refraction and corneal curvature of the eye.[24] The AVR was defined as the ratio of the summated arteriolar-to-venular diameters.

A random sub-sample of 40 transparancies was used to monitor quality of the data at regular intervals. Pearson's correlation coefficients for intergrader agreement were 0.67-0.80 (arteriolar diameter), 0.91-0.94 (venular diameter) and 0.75-0.84 (AVR). For intragrader agreement these figures were 0.69-0.88, 0.90-0.95 and 0.72-0.90 respectively.

**Other variables**

Atherosclerotic plaques were assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides and defined as focal thickening of the vessel wall (of at least 1.5 times the average intima-media thickness) relative to adjacent segments with or without calcified components. The carotid artery plaque score (range:0-6) reflects the number of these locations with plaques.[25] Non-fasting serum total and HDL cholesterol concentration was determined by an automated enzymatic procedure.[26] Height and weight were measured and the body mass index (BMI) was calculated by dividing body weight (kg) by height squared ($m^2$). Leukocyte count was assessed in citrate plasma using a Coulter Counter T540® (Coulter electronics, Luton, England). Blood was drawn directly into VACUTAINER® tubes and

erythrocyte sedimentation rate was read after 60 minutes. Serum levels of C-reactive protein (CRP) were determined by the Rate Near Infrared Paricle Immunoassay method (Immage® high sensitive CRP, Beckman Coulter, USA). Smoking habits (categorized as current, former and never smoking) and use of anti-hypertensive medication was assessed during the baseline interview. Diabetes mellitus was considered present if participants reported use of antidiabetic medication or when random or post-load serum glucose level was greater than 11 mmol/l. Blood pressure was measured twice with a random zero sphygmomanometer at the brachial artery with the subject in sitting position, and the measurements were averaged. Apolipoprotein-E (APOE) genotyping was performed on coded DNA samples without knowledge of the diagnosis. The polymerase chain reaction product was digested with the restriction enzyme *HhaI*, and fragments were separated by electrophoresis.[27]

**Statistical analysis**

Analysis of covariance (ANCOVA), adjusted for age and gender, was used to compare baseline characteristics of participants with and without gradable fundus transparencies. Associations between baseline retinal vessel diameters and incidence of dementia and its major subtypes Alzheimer's disease and vascular dementia were assessed with Cox' proportional hazards models. Hazard ratios (HR) with corresponding confidence intervals (CI) were adjusted for age and gender. Age$^2$ was entered to adjust for potential residual confounding by age. Participants were followed either until onset of dementia, death, or the end of the study. Age at onset of dementia was determined as the age at midpoint between the date at which the dementia diagnosis was made and the last center visit. Retinal arteriolar and venular diameters and AVR were first entered in quintiles to check whether the relation with dementia was linear. The associations did not show large deviations from linearity and thus all analyses were subsequently performed entering retinal vessel diameters and AVR as a linear term in the model. HRs are expressed per standard deviation (SD) difference in retinal vessel diameter or AVR to allow comparison of strength of associations across the different vessel characteristics. The AVR is presented to compare our results with other studies.

Additional adjustments were made for carotid artery plaque score, serum levels of total and HDL cholesterol, body mass index, C-reactive protein, smoking, diabetes mellitus, systolic and diastolic blood pressure, antihypertensive treatment. To evaluate whether relations were different in diabetics or differed according to APOE status, analyses were repeated after

stratification for diabetes mellitus and APOE. All analyses were performed using SPSS statistical software version 11 (SPSS Inc., Chicago, Illinois).

## RESULTS

Fundus transparancies were gradable in at least one eye in 5553 (89.3%) of the 6219 participants who underwent the eye examination at baseline. The remaining 666 participants had ungradable fundus transparencies on both eyes. Age and gender adjusted differences show that those excluded were significantly older, more often institutionalized and had slightly higher blood pressures. There were no significant differences in other risk factors. The mean arteriolar diameter was 147.0 μm (standard deviation (SD): 14.4 μm; range 92.2-235.7 μm) venular diameter 222.2 μm (SD: 20.8 μm; range 135.1-313.6 μm) and arteriolar-to-venular ratio 0.66 (SD: 0.06; range 0.48-1.02). After a follow-up of 32,950 person-years (mean: 5.9 years (SD: 1.4)), 233 participants had developed dementia, of whom 167 had Alzheimer's disease and 38 vascular dementia.

Table 2 shows that per SD increase in venular diameter the risk of

**Table 1.** Baseline characteristics of study participants with gradable or ungradable fundus transparencies presented as unadjusted means (SD) or percentages

| | Gradable* | Ungradable | Adjusted differences† (95 % CI)‡ |
|---|---|---|---|
| Number (n) | 5553 | 666 | |
| Age (years) | 67.7 (8.0) | 74.5 (9.8) | 6.7 (6.0; 7.4) |
| Gender (% female) | 58.6 | 60.8 | -1.4 (-5.0; 3.0) |
| Institutionalized (%) | 2.8 | 15.2 | 4.3 (2.5; 6.1) |
| Number of carotid artery plaques ≥ 4 (%) | 16 | 22 | 0.6 (-2.7; 4.0) |
| Serum total cholesterol (mmol/l) | 6.64 (1.21) | 6.59 (1.28) | 0.05 (-0.05; 0.15) |
| Serum HDL cholesterol (mmol/l) | 1.35 (0.36) | 1.34 (0.36) | 0.01 (-0.02; 0.04) |
| Body mass index (kg/m$^2$) | 26.3 (3.7) | 26.1 (3.7) | -0.3 (-0.6; -0.2) |
| Smoking (% current) | 23.7 | 19.6 | -2.5 (-5.9; 1.0) |
| C-reactive protein (mg/L) | 3.15 (6.08) | 4.10 (11.0) | 0.49 (-0.07; 1.05) |
| Diabetes mellitus (%) | 9.5 | 13.7 | 1.3 (-1.1; 3.8) |
| Systolic blood pressure (mmHg) | 138.4 (22.0) | 145.3 (23.8) | 2.4 (0.6; 4.2) |
| Diastolic blood pressure (mmHg) | 73.7 (11.3) | 74.2 (12.4) | 1.4 (0.4; 2.3) |

* Participants with a gradable fundus transparency on at least one eye
† age and gender adjusted if applicable
‡ CI: confidence interval

**Table 2.** Hazard Ratios* of incident dementia and its major subtypes per SD difference in retinal vessel diameters or arteriolar-to-venular ratio

| | Dementia (n=233) | Alzheimer's disease (n=167) | Vascular dementia (n=38) |
|---|---|---|---|
| Arteriolar narrowing† | 0.94 (0.83; 1.06) | 0.90 (0.78; 1.04) | 1.09 (0.79; 1.50) |
| Venular dilatation‡ | 1.21 (1.07; 1.37) | 1.22 (1.06; 1.41) | 1.21 (0.89; 1.64) |
| Arteriolar-to-venular ratio§ | 1.14 (1.00; 1.29) | 1.09 (0.94; 1.27) | 1.35 (0.98; 1.87) |

* Hazard ratios (95% confidence interval) adjusted for age and gender
† Hazard ratio per SD decrease in retinal arteriolar diameters
‡ Hazard ratio per SD increase in retinal venular diameters
§ Hazard ratio per SD decrease in arteriolar-to-venular ratio

incident dementia increased significantly by 21%. Smaller arteriolar diameters were not associated with an increased risk of dementia, whereas each SD decrease in AVR increased risk of dementia by 14%. Associations for the major subtypes Alzheimer's disease and vascular dementia were similar: per SD increase in venular diameter the risk of Alzheimer's disease and vascular dementia increased by 22 and 21% respectively. However, for the latter this was not significant. Arteriolar diameters were not associated, whereas a lower AVR was non-significantly associated with an increased risk of both Alzheimer's disease and vascular dementia.

Participants in the highest quintile of venular diameters had a 98% increased risk of dementia compared to those in the lowest quintile (table 3). Compared with participants in the lowest four quintiles of venular diameters those in the highest quintile had a significantly increased risk of dementia of 73%. Quintiles of arteriolar diameters and AVR were not related to the risk of dementia (table 3).

In table 4 the association between venular diameters and dementia and its major subtypes are presented after additional adjustment for other cardiovascular risk factors. The strenghts of the associations hardly changed after these additional adjustments. In the fully adjusted model each SD increase in venular diameter the risk of dementia increased by 23%. For Alzheimer's disease and vascular dementia the corresponding risks increased by 25% and 14% respectively. For vascular dementia this was non-significant. The association between arteriolar diameters and dementia also remained unchanged after additional adjustment for other cardiovascular risk factors (HR per SD decrease in arteriolar diameter: 0.93; 95% CI: 0.80; 1.09). The association with AVR was somewhat attenuated after additional adjustment (HR per SD decrease in AVR: 1.12; 95% CI: 0.96; 1.31) and became non-

**Table 3.** Hazard ratios* of incident dementia and its major subtypes per quintile [intra-quintile range] of baseline retinal vessel diameters or arteriolar-to-venular ratio

| | **Dementia (n = 233)** | **Alzheimer's disease (n = 167)** | **Vascular dementia (n=38)** |
|---|---|---|---|
| Arteriolar diameter (μm) | | | |
| 5. [158.2-235.7] | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) |
| 4. [149.7-158.2] | 1.37 (0.90; 2.07) | 1.25 (0.77; 2.04) | 1.06 (0.38; 2.92) |
| 3. [143.1-149.7] | 1.12 (0.72; 1.72) | 1.13 (0.69; 1.87) | 0.66 (0.21; 2.07) |
| 2. [135.3-143.1] | 1.09 (0.71; 1.67) | 1.20 (0.74; 1.95) | 0.63 (0.20; 2.00) |
| 1. [92.2-135.3] | 0.89 (0.58; 1.37) | 0.73 (0.43; 1.23) | 1.44 (0.57; 3.62) |
| Q1 versus Q2-5† | 0.78 (0.56; 1.08) | 0.63 (0.42; 0.95) | 1.73 (0.88; 3.38) |
| Venular diameter (μm) | | | |
| 1. [135.1-204.5] | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) |
| 2. [204.5-216.3] | 1.32 (0.89; 1.97) | 1.82 (1.14; 2.90) | 0.28 (0.08; 0.99) |
| 3. [216.3-226.9] | 1.00 (0.65; 1.56) | 1.46 (0.88; 1.56) | 0.30 (0.09; 1.06) |
| 4. [226.9-239.1] | 1.28 (0.84; 1.94) | 1.66 (1.01; 1.94) | 0.32 (0.09; 1.14) |
| 5. [239.1-313.6] | 1.98 (1.34; 2.93) | 1.97 (1.19; 2.93) | 1.78 (0.84; 3.79) |
| Q5 versus Q1-4‡ | 1.73 (1.28; 2.34) | 1.35 (0.91; 2.00) | 3.54 (1.84; 6.80) |
| Arteriolar-to-venular ratio | | | |
| 5. [0.71-1.02] | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) |
| 4. [0.68-0.71] | 0.95 (0.62; 1.47) | 0.97 (0.59; 1.59) | 0.97 (0.33; 2.88) |
| 3. [0.65-0.68] | 1.19 (0.79; 1.79) | 1.16 (0.72; 1.86) | 0.97 (0.32; 2.88) |
| 2. [0.62-0.65] | 1.49 (0.99; 2.23) | 1.42 (0.88; 2.28) | 1.42 (0.51; 3.94) |
| 1. [0.48-0.62] | 1.37 (0.92; 2.04) | 1.34 (0.84; 2.13) | 1.75 (0.67; 4.53) |
| Q1 versus Q2-5§ | 1.20 (0.88; 1.64) | 1.20 (0.83; 1.73) | 1.62 (0.80; 3.62) |

* Hazard ratios (95% confidence interval) were adjusted for age and gender
† Lowest versus all other quintiles of retinal arteriolar diameters
‡ Highest versus all other quintiles of retinal venular diameters
§ Lowest versus all other quintiles of retinal arteriolar-to-venular ratio

significant. Associations between arteriolar diameters or AVR and the subtypes Alzheimer's disease and vascular dementia were similar to dementia. Exclusion of participants with diabetes did not change the results, nor did stratification on APOE*4.

## DISCUSSION

The results show that larger venular diameters are associated with an increased risk of dementia. This association was similar for the major subtypes Alzheimer's disease and vascular dementia. These associations were independent of traditional vascular risk factors.

Important advantages of our study are the population-based design and the long follow-up, which with regard to the dementia diagnosis was virtually complete. Other advantages of our study are the detailed assessment of vessel diameters on 20° stereoscopic transparencies obtained after pharmacological mydriasis and the use of Littmann's correction to adjust for refractive errors of the eye. This enabled us to obtain approximated absolute intra-luminal arteriolar and venular diameters, where others reported uncorrected vessel diameters in pictures with smaller magnification.[28,29]

Retinal venular diameters may be related to dementia in several ways. Larger venular diameters have been related to markers of inflammation, more sub-clinical atherosclerosis, and higher total and lower HDL cholesterol.[13] Our finding that larger venular diameters increase risk of dementia might thus be explained by inflammation or atherosclerosis. Adjustment for either markers of inflammation or sub-clinical atherosclerosis did not change our results however. In addition, venular widening has been observed in the early stages of diabetic retinopathy.[29] As diabetes mellitus has been associated with dementia,[30] the association between venular diameter and dementia may reflect

**Table 4.** Hazard ratios* of dementia and its major subtypes per standard deviation increase in retinal venular diameters additionally adjusted for other cardiovascular risk factors

| | Dementia (n = 233) | Alzheimer's disease (n = 167) | Vascular dementia (n=38) |
|---|---|---|---|
| Age and gender | 1.21 (1.07; 1.37) | 1.22 (1.06; 1.43) | 1.21 (0.89; 1.50) |
| Age, gender and carotid artery plaque score | 1.25 (1.09; 1.43) | 1.27 (1.09; 1.43) | 1.10 (0.79; 1.54) |
| Age, gender, total and HDL cholesterol | 1.21 (1.07; 1.37) | 1.22 (1.05; 1.41) | 1.18 (0.87; 1.61) |
| Age, gender and body mass index | 1.21 (1.07; 1.38) | 1.23 (1.06; 1.42) | 1.21 (0.89; 1.64) |
| Age, gender, and CRP† | 1.21 (1.04; 1.39) | 1.23 (1.04; 1.46) | 1.20 (0.82; 1.75) |
| Age, gender and smoking | 1.23 (1.08; 1.39) | 1.22 (1.05; 1.41) | 1.29 (0.94; 1.76) |
| Age, gender and diabetes mellitus | 1.21 (1.07; 1.37) | 1.22 (1.06; 1.41) | 1.20 (0.88; 1.64) |
| Age, gender, blood pressure and medication‡ | 1.21 (1.07; 1.37) | 1.23 (1.06; 1.42) | 1.18 (0.87; 1.61) |
| Fully adjusted | 1.23 (1.05; 1.44) | 1.25 (1.04; 1.50) | 1.14 (0.74; 1.77) |

* Hazard ratios (with corresponding 95% confidence interval)

† CRP: C-reactive protein

‡ Systolic and diastolic blood pressure and anti-hypertensive medication

diabetes. Additional exclusion of participants with diabetes mellitus did not alter our results, indicating that the association was not confined to participants with larger venular diameters due to diabetic retinopathy. Adjustment for other vascular risk factors did not change our results, indicating that other mechanisms should be considered.

Venular widening has also been observed in venous stasis retinopathy, a condition due to carotid artery obstruction and associated with impaired flow to both the retinal and the cerebral circulation.[31] We hypothesized that larger venular diameters might reflect disturbed blood flow, leading to cerebral hypoperfusion and ischaemia and as such contribute to dementia and Alzheimer's disease. It has been suggested that advanced aging in combination with different vascular risk factors leads to progressive degeneration of the microvasculature of the brain, which subsequently gives rise to chronic disturbed blood flow and cerebral hypoperfusion, impairment of the delivery of essential nutrients to cerebral neurons, reactive astrocytosis and finally plaques and tangle formation.[32] Thus far, only a few studies investigated the role of cerebral venular abnormalities in dementia and brain aging. One study reported wall thickening and obstruction of periventricular veins, referred to as periventricular venous collagenosis, in brains with severe white matter lesions.[33,34] These changes have been suggested to increase venous pressure and subsequently give rise to venular dilatation and venular blood-brain barrier disruption. Venous stasis could in turn lead to cerebral hypoperfusion and ischemia in the periventricular region through diminished clearance of cellular metabolites and as such contribute to the development of white matter lesions and dementia.[34,35] In this population-based study brain imaging was not performed routinely in all participants. Therefore we were not able to investigate whether white matter lesions could account for the association we found between venular diameters and risk of dementia. Moreover it is not known to what extent retinal venular diameters reflect cerebral venular diameters and the pathological events leading to venular widening in the retina still need to be elucidated.

Whereas larger venular diameter increased the risk, arteriolar diameter was not associated with the risk of dementia or its major subtypes. Smaller arteriolar diameters have been related to higher blood pressures[13,36] and both high and low blood pressures have been found to increase risk of dementia.[37,38] An association between arteriolar diameter and risk of dementia might therefore be expected. However, risk of dementia is known to increase especially at higher age.[15] The association between arteriolar diameter and blood pressure on the other hand becomes gradually weaker with advancing

age as retinal arterioles become progressively rigid and lose their ability to react to blood pressure changes due to vasoconstriction, intimal thickening, medial hyperplasia, hyalinisation and sclerosis of the vessel wall.[7,39] Due to this increased stiffness of the arterial vessel wall at higher age, widening of retinal arterioles on the other hand may be less pronounced than in retinal venules in conditions reflecting impaired flow and ischaemia to the retina and the brain. This is supported by the fact that venular widening instead of arteriolar widening is more strongly associated with conditions reflecting ischaemia to the retina or the brain, including diabetic retinopathy, incidence of proliferative diabetic retinopathy and carotid artery occlusion.[29,31,40]

In our study a lower AVR tended to be associated with a higher risk of dementia and its major subtypes. This seems in correspondence with previous findings relating a lower AVR to a higher risk of stroke.[11] Yet, a lower AVR was not related to cognitive performance and cerebral atrophy on MRI of the brain.[12,41] Until recently only the AVR was presented, in which the arterioles are gauged against the venules to correct for refractive errors of the eye. This was thought acceptable since venular diameters showed only weak associations with blood pressure and age.[8] The AVR as a ratio is however conceptually difficult since lower AVR may be due to both arteriolar narrowing and venular widening. Moreover, arterioles and venules respond differently to various pathological conditions including hypertension, subclinical atherosclerosis and diabetic retinopathy.[8,13,40] This may explain why findings relating the AVR to cerebrovascular disease are inconsistent. The abovementioned findings imply that at least in our study the association between a lower AVR and risk of dementia is due to larger venular diameters instead of smaller arteriolar ones.

Several methodological considerations need to be discussed. Participants excluded due to poor quality of fundus transparancies were on average older and more often institutionalized. However, since the two groups hardly differed in any of the other cardioavscular risk factors, we do not think this has introduced selection bias. Limitations related to the semi-automated system used to assess the retinal vessel diameters, have been described.[8] For instance, photographs were taken independent of the cardiac cycle and thus effects of pulsatility on vessel width cannot be ruled out. Most likely these limitations led to an underestimation of our effects due to random misclassification because assessment of vessel diameters was unrelated to clinical characteristics of the participants.

In conclusion we found larger retinal venular diameters to be associated with an increased risk of dementia and its major subtypes Alzheimer's disease

and vascular dementia independent of other vascular risk factors. Our finding adds to the evidence suggesting that vascular factors are of importance not only in the pathogenesis of vascular dementia but also Alzheimer's disease. Moreover, this is the first study to suggest a role of pathology associated with larger retinal venular diameters in the etiology of dementia including Alzheimer's disease. Further research needs to be done to confirm and clarify this association and the eventual clinical relevance of our finding.

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# Chapter 4.4

# Aging Macular Disease in relation to Alzheimer's Disease and Brain Atrophy on MRI


## ABSTRACT

*The authors studied aging macular disease (AMD) prospectively in relation to the risk of Alzheimer's disease (AD) and cross-sectionally in relation to structural brain changes on magnetic resonance imaging (MRI). This study was performed as part of the Rotterdam Study, a prospective population-based cohort study, which was initiated in 1990-1993. At baseline 6200 participants, aged 55 years or older, were screened for the presence of AMD and AD; followed by follow-up screenings in 1993-1994 and 1997-1999. Furthermore, in 1995 to 1996 a sample of 442 non-demented elderly underwent cerebral MRI scanning. Structural brain changes included hippocampal, amygdalar and generalized brain atrophy, which may be considered as preclinical markers of AD. Persons with baseline AMD were not at an increased risk of AD (hazard ratio = 0.97, 95% confidence interval: 0.66, 1.43; adjusted for age and gender). In line with these observations, no relation was found between AMD and measures of brain atrophy. These data do not support our previous notion that AMD is an ocular manifestation of AD.*


Submitted

The neuropathology in Alzheimer's disease (AD) includes neuronal loss, neurofibrillary tangles and amyloid plaques, and is most marked in the temporo-parietal areas resulting in memory deficits and cognitive decline (1). Some patients, however, may have prominent visual deficits (2). Although these symptoms are mainly attributed to the presence of AD-related neuropathology in the central visual system (2), the retina may also show ganglion cell and axonal degeneration in AD (3). Drusen, extra-cellular deposits presumably derived from the degenerating photoreceptors and retinal pigment epithelium, are an early sign of age-related macular degeneration or, as we prefer to call it, aging macular disease (AMD) (4). Based on similarities between these extra-cellular deposits, several studies linked AMD to AD, but the evidence is inconclusive (5, 6). In the Rotterdam Study, preliminary data suggested that AMD predicted the two-years risk of AD in participants above 75 years (5). However, this relation was partially explained by shared cardiovascular risk factors (5). We now have a longer follow-up and re-examined this association. Moreover, we investigated if there was clustering of these two diseases within a person, irrespective of AMD preceding AD or vice versa.

To further explore this relation, we examined cross-sectionally a

possible association between AMD and structural brain changes on magnetic resonance imaging (MRI) including hippocampal, amgydalar and generalized brain atrophy, which may be considered as preclinical markers of AD (7). We investigated this in a subset of the Rotterdam Study, that participated in the population-based Rotterdam Scan Study (7).

## MATERIALS AND METHODS

### Study population

The present study was performed as part of the Rotterdam Study, a prospective, population-based cohort study of persons aged 55 years or older (8). The study was conducted according to the Declaration of Helsinki, and the Medical Ethics Committee of the Erasmus Medical Center approved the study protocol. All participants gave written informed consent. Baseline examinations were performed from 1990 to 1993, followed by follow-up screenings in 1993-1994 and 1997-1999. Of 6780 participants, who underwent an ophthalmologic examination, 6418 persons had gradable fundus transparencies necessary for the diagnosis of AMD at baseline (5). Gradable transparencies were available of 4974 persons at the first, and of 3636 at the second follow-up examination. After excluding 218 persons with prevalent dementia (including 144 with prevalent AD) the cohort at risk for dementia consisted of 6200 participants.

Furthermore, in 1995 to 1996 we randomly selected 1077 non-demented participants (60-90 years) in strata of gender and 5-year age groups from two ongoing population-based studies; MRI images were obtained on 563 participants from the Rotterdam Study. Of these 442 persons had gradable fundus transparencies, taken during the second follow-up examination.

### Aging macular disease grading

A detailed description of the diagnostic procedures has been presented elsewhere (9). Participants underwent a full eye examination, including stereo 35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) centered on field 2 (the fovea) following pharmacological mydriasis. The resulting transparencies were graded with 12.5x magnification according to the International Classification and Grading System for AMD (5). Two well-trained graders, each having eight years of experience, graded the fundus transparencies masked for all other participant characteristics. The grading procedures and definitions, as well as the graders, were identical at baseline and at follow-up.

Early AMD was defined as presence of either soft distinct drusen with pigmentary irregularities, or indistinct or reticular drusen with or without pigmentary irregularities; late AMD as atrophic or neovascular end-stages. Consensus sessions and inter-grader comparisons were performed regularly. Weighted kappa values were 0.78 for drusen type, 0.80 for hyperpigmentation, 0.58 for hypopigmentation, and 0.84 for late AMD (10).
Per person, both eyes were classified as no, early or late AMD. For this analysis a person was classified according to the eye with the most advanced stage of AMD.

## Dementia screening

Dementia screening both at baseline and follow-up followed a three-step protocol (5). After an initial cognitive screening (Mini-Mental State Examination and the Geriatric Mental State schedule, organic level), screen positives underwent further cognitive testing, and an informant interview was obtained. Persons suspected of dementia were examined by a neurologist and underwent more elaborate neuropsychological testing. In addition, the entire cohort was monitored for incident dementia via computerized linkage of the study database with medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. Surveillance of the population continued till December 1999. Diagnoses of dementia and its

**Table 1**. Characteristics of the total study sample at baseline (1990-1993) and its subset with MRI examination (at time of MRI: 1995-1996) presented as means or percentages

| | Rotterdam Study | Subset with MRI |
|---|---|---|
| Number of participants | 6200 | 442 |
| Age (years) | 68.5 (8.5)* | 72.4 (7.6)* |
| Gender (% women) | 58.9 | 50.0 |
| AMD†: Early | 448 | 50 |
| Late | 89 | 10 |
| Incident dementia | 294 | - |
| Incident Alzheimer's disease | 196 | - |
| Hippocampal volume (ml) | - | 6.43 (0.87)* |
| Amygdalar volume (ml) | - | 4.61 (0.72)* |
| Cortical atrophy (sum grade: 0-15) | - | 5.86 (3.0)* |
| Subcortical atrophy (ratio) | - | 0.31 (0.04)* |

* Standard deviation
† AMD = aging macular disease

major subtype AD were made on all existing information by an expert panel consisting of a neurologist, neuropsychologist and research physician, and in accordance with internationally accepted criteria (5).

**Quantification of brain atrophy**

Standard T1-, T2- and proton density-weighted axial scans and a custom-made three-dimensional MRI sequence covering the whole brain were made using a 1.5-T MR unit (MRVISION, Siemens, Germany) (7).

Each contiguous 1.5-mm thick coronal brain slice from the three-dimensional MRI was manually traced with a mouse-driven cursor to measure the left and right hippocampus and amygdala. Absolute volumes (ml) were calculated by multiplying the areas by slice thickness and left and right sides were summed. We also corrected for head size differences by using the intracranial cross-sectional area, measured on the middle sagittal slice (7).
As the volumetric assessment was time-consuming, the scans were equally distributed between two graders. Intra- and intergrader studies based on 14 scans done by both graders showed intraclass correlation coefficients to exceed $r = 0.77$.

We rated cortical atrophy semi-quantitatively on axial T1 MRI slices. Two graders scored independently all the scans and gave a score of 0 (no cortical atrophy) to 3 (severe cortical atrophy) at frontal, temporal, parietal, occipital and insular regions based on the size of sulci and gyri. The sum grade (range: 0-15) was indicative of generalized cortical atrophy (7). In case of disagreement of more than one point between the graders a consensus reading was held, otherwise the mean score was calculated. Weighted kappa values of intra- and intergrader agreement were 0.82 and 0.81 based on 200 scans. Subcortical atrophy was measured as the ventricle-to-brain ratio

**Table 2.** Age and gender adjusted hazard ratios (95% CI)* of dementia and Alzheimer's disease for persons with prevalent aging macular disease

| | Persons at risk | Dementia | HR (95% CI)* | AD | HR (95% CI)* |
|---|---|---|---|---|---|
| No AMD† | 5663 | 243 | 1.0 (reference) | 162 | 1.0 (reference) |
| AMD | 537 | 51 | 0.99 (0.73-1.35) | 34 | 0.97 (0.66-1.43) |
| Early AMD | 448 | 36 | 0.91 (0.64-1.30) | 23 | 0.87 (0.55-1.35) |
| Late AMD | 89 | 15 | 1.28 (0.75-2.18) | 11 | 1.36 (0.72-2.55) |

* HR (95% CI): hazard ratios (with corresponding 95% confidence interval)
† AMD = aging macular disease
‡ AD = Alzheimer's disease

**Table 3**. Quantified brain atrophy on MRI according to presence of aging macular disease, presented as means (± standard errors) adjusted for age and gender

| | **Hippocampus (ml)** | **Amygdala (ml)** | **Cortical atrophy (grade)** | **Sub-cortical atrophy†** |
|---|---|---|---|---|
| No AMD* (382) | 6.41 ± 0.05 | 4.61 ± 0.04 | 5.90 ± 0.13 | 0.306 ± 0.002 |
| Early AMD (50) | 6.56 ± 0.12 | 4.64 ± 0.10 | 5.56 ± 0.36 | 0.304 ± 0.005 |
| Late AMD (10) | 6.32 ± 0.27 | 4.51 ± 0.22 | 6.03 ± 0.80 | 0.313 ± 0.011 |
| Test for trend: p-value | 0.49 | 0.85 | 0.66 | 0.70 |

* AMD = aging macular disease
† ventricle-to-brain ratio

(average of assessments at three locations).

**Statistical analyses**

To assess whether baseline AMD predicted incident dementia and AD, Cox proportional hazards models were used. Next, for the clustering analysis, we pooled all prevalent and incident cases both for AMD and AD, and assessed if persons with AMD were more likely to have AD. For participants who did not have AMD data at the second follow-up examination, we used AMD and AD data available at the first follow-up examination. For those, who had only baseline AMD data, their baseline AD status was taken. For this clustering analysis, logistic regression models were used. Finally, the cross-sectional association between AMD and brain atrophy was studied using analysis of covariance. We used SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

Table 1 shows the general characteristics of the study sample at baseline and of the subset at time of MRI. After a mean follow-up of 5.8 years, 294 persons developed dementia, of whom 196 had AD. We found no evidence for an increased risk of dementia or its major subtype AD among persons with baseline AMD, neither for early nor for late stages of AMD (table 2). For the clustering analysis, 929 persons with AMD (541 prevalent, 388 incident) and 225 with AD (144 prevalent, 81 incident) were available. Persons with AMD were no more likely to have AD than persons without (odds ratio: 1.03, 95% confidence interval: 0.74, 1.43; adjusted for age and gender). In line with these observations, no relation was found between AMD and different measures of brain atrophy (table 3).

## DISCUSSION

In this prospective population-based study with an average follow-up of almost six years, we could not confirm our previous findings that baseline AMD was related to an increased risk of AD. Furthermore, there was no clustering of AMD and AD. Finally, we could not find a relation between AMD and brain atrophy on MRI.

The reverse association between baseline AD and incident AMD could not be assessed, because in only 30% of the participants with the prevalent AD fundus photographs were available at follow-up.

Until now, evidence for a direct link between AMD and AD has been inconclusive. Although amyloid-associated proteins tau and amyloid precursor protein are expressed in the retina (11), immunoreactivity against tangles or amyloid plaques has not been consistently found in drusen (12). Also, histopathological studies on retinal ganglion cell and axonal degeneration in the macula and optic disc in AD have not provided a decisive answer (2, 3, 13). These discrepancies have been attributed to differences in methodology such as effects of tissue processing on drusen composition, postmortem delay in axon count, cell identification criteria, and sample sizes (12).

We know of only one other study that examined the relation between AMD and cognitive dysfunction. In a cross-sectional analysis, it was found that persons who performed worse on a Word Fluency Test were more likely to have early AMD (odds ratio: 1.6, 95% confidence interval: 1.1, 2.3) (6). However, other neuro-psychological tests were not related to AMD (6). In this current report, we showed that AMD was neither related to AD, nor to brain atrophy on MRI. Our data did not provide evidence for AMD being an ocular manifestation of AD.

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# *Part V*

# *Cardiovascular Epidemiology*

# Chapter 5.1

# Retinal Vessel Diameters and Risk of Hypertension: The Rotterdam Study


## ABSTRACT

*Generalised arteriolar narrowing is an important feature of systemic hypertension. A lower retinal arteriolar-to-venular diameter ratio reportedly predicts the risk of hypertension. This suggests that narrowing may already precede hypertension rather than occurs as a secondary adaptation. We investigated if retinal arteriolar and venular diameters separately were related to the risk of hypertension.*

*In the prospective population-based Rotterdam Study (1990-1993), baseline retinal arteriolar and venular diameters and their ratio (AVR) were measured on digitised images of one eye of 1900 normotensive participants aged 55 years or older. Blood pressure data obtained at the follow-up examination (1997-1999) were used to detect cases with incident hypertension, defined as either systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg, or use of medication for the treatment of hypertension.*

*After a mean follow-up of 6.6 years, 808 persons developed hypertension. Adjusted for age, gender, follow-up time, body mass index, smoking, diabetes mellitus, total and high-density lipoprotein cholesterol, C-reactive protein and intima-media thickness, arteriolar narrowing was associated with an increased risk of hypertension (OR per standard deviation (SD): 1.38 [1.23-1.55]), as was venular narrowing (OR per SD: 1.17 [1.04-1.32]). Each SD decrease in the AVR significantly increased the risk of hypertension by 24%. To examine the effect of baseline blood pressure, we stratified persons into "optimal" (systolic < 120 and diastolic < 80) or "normal/borderline" (systolic 120-139 or diastolic 80-90) blood pressure. Within these strata, arteriolar narrowing was still related to incident hypertension.*

*These data provide further evidence that retinal arteriolar narrowing may precede the development of systemic hypertension.*


Submitted

An increased peripheral vascular resistance, a hallmark of hypertension, is mainly determined by arterioles.[1-3] It remains uncertain whether generalised arteriolar narrowing antedates high blood pressure or occurs as a secondary adaptation.[2] A primary role has been attributed to impaired renal sodium homeostasis leading to fluid expansion and subsequently to increased cardiac output and blood pressure.[3,4] According to this hypothesis, physiological and morphological alterations in the peripheral circulation arise as a secondary reaction.

In recent years, however, other hypotheses, postulating alterations in arterioles as the initiating event in the development of hypertension, have gained support.[2] Thus far, evidence is largely based on animal models.[2,5] Spontaneously hypertensive rats already had decreased arteriolar lumina compared to control rats prior to the onset of hypertension.[6,7] Studies in humans suggested that persons with familial predisposition had reduced arteriolar vasodilatation in the pre-hypertensive stage.[8]

A semi-automated system was developed to measure non-invasively retinal vessel diameters in vivo.[9] A lower arteriolar-to-venular ratio (AVR) thus obtained was suggested to reflect generalized arteriolar narrowing and was associated with an increased risk of developing hypertension within three years.[10] We recently showed that venular diameters do vary with several

cardiovascular risk factors, making it difficult to interpret the AVR.[11] Thus we proposed to study arteriolar and venular diameters separately, especially in etiological research. So far, prospective data on the relationship between retinal arteriolar diameters and hypertension are scarce. We examined the relation between baseline retinal vessel diameters and incident hypertension in a prospective population-based setting.

## METHODS

### Study population

This study was performed as part of the Rotterdam Study, a prospective population-based cohort study on chronic diseases in the elderly.[12] A total of 7983 participants, aged 55 years or older, living in a district of Rotterdam agreed to participate in the study (response rate 78%). Because the ophthalmic part became operational after the screening of at random invited participants had started, a smaller number (n = 6780) participated in the eye examination. The study was conducted according to the tenets of the Declaration of Helsinki and the medical ethics committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline interviews and examinations were performed from 1990 to 1993, followed by follow-up examinations, the last one from 1997 to 1999.

### Retinal vessel measurements

Simultaneous stereoscopic fundus colour transparencies centered on the optic disc (pharmacological mydriasis, $20^0$ field, Topcon Optical Company, Tokyo, Japan) were taken at the baseline eye examination. These transparencies were digitised with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan) and for each participant the image of one eye with the best quality was analysed with a semi-automated system (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison) by one of four trained graders masked for all participant characteristics.

We used the improved Parr-Hubbard formula to compute the summary vessel measures.[11] Because eyes may have a different magnification due to refractive errors we additionally adjusted these summary vessel measures.[11] On one eye of each participant one sum value was calculated for the arteriolar blood column diameter and one for the venular (in μm), the AVR being the ratio of these. In a random sub-sample of 100 participants

we found no differences between the right and left eyes for the arteriolar and venular diameters.

**Ascertainment of incident cases of hypertension**

Both at the baseline and follow-up examinations, trained examiners took two blood pressure measurements with a random-zero sphygmomanometer with appropriate adult cuff size in sitting position. The average of these two measurements was taken. Persons with a systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg were considered to be normo-tensive.[13,14] Stage 1 hypertension was defined as either systolic blood pressure 140 to 159 mmHg, or diastolic blood pressure 90 to 99 mmHg, or both. Stage 2 hypertension was defined as either systolic blood pressure ≥ 160, or diastolic blood pressure ≥ 100, or both, or use of anti-hypertensive medication. Exposure to medication was assessed at baseline and follow-up by means of a standardized interview. Participants were asked to bring their medications to the examination center to verify the names of medications they were taking. Anti-hypertensive agents included angiotensin converting enzyme inhibitors, calcium channel blockers, beta-blockers (excluding topical medications), diuretics, and other anti-hypertensive agents (such as centrally acting anti-hypertensive drugs). Incident hypertension was defined as being normotensive at baseline and the presence of hypertension stage 1 or 2 at the follow-up examination. We also repeated the analyses with a stricter definition: being normotensive at baseline and developing hypertension stage 2 during follow-up.

**Assessment of confounders**

All information regarding confounders was assessed at the baseline examination. Information on smoking (categorized as current, former or never) was obtained during the home interview. Body mass index was computed as weight divided by height squared. Non-fasting serum total cholesterol was determined by an enzymatic procedure and high-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[15] Diabetes mellitus was considered present if participants reported use of anti-diabetic medication or when random or post-load serum glucose level was > 11 mmol/l. Intima-media thickness (in mm) was ultra-sonographically measured in the common carotid arteries.[16] Serum levels of C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Immage® high sensitive CRP, Beckman Coulter, USA).

**Study sample**

Of the 6780 participants in the ophthalmic part of the Rotterdam Study, 6436 persons had optic disc photographs and in 5674 (84%) persons these transparencies were gradable for retinal vessel measurements. Excluding participants who had missing data on blood pressure (n = 84) and those with prevalent hypertension (n = 2871), a total of 2719 participants remained at risk for incident hypertension. During follow-up 317 participants died, a further 502 refused or were unable to participate at the follow-up examination leaving 1900 normotensive participants for the current analyses.

**Statistical analyses**

We calculated the odds ratios (OR) with corresponding 95% confidence intervals (CI) of incident hypertension per standard deviation (SD) difference in retinal vessel diameters by using logistic regression analysis, adjusted for age, gender and follow-up time. Additional adjustments were made for baseline levels of smoking, body mass index, diabetes mellitus, total and HDL cholesterol, CRP and atherosclerosis as measured by the intima-media thickness. Subsequently, the analyses were repeated by categorizing the retinal vessel diameters into quartiles of their distribution.

In order to examine whether the association between retinal vessel diameters and incident hypertension was confounded by baseline blood pressure, we decided to stratify baseline blood pressure into two subcategories, as suggested by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-VII).[14] An optimal blood pressure was defined as systolic blood pressure < 120 mmHg and diastolic blood pressure < 80 mmHg. A systolic blood pressure of 120 to 139 or diastolic blood pressure of 80 to 89 was considered to be normal to borderline. Within these strata, we again analyzed the association between baseline retinal vessel diameters and incident hypertension.

Finally, the analyses were repeated using our stricter definition of stage 2 incident hypertension. Those who developed stage 1 hypertension were excluded from this analysis. All analyses were performed with SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

## RESULTS

Table 1 presents the baseline characteristics of our study population. Non-participants were older, more often women, more often current smokers and had a slightly higher CRP level compared to participants. After a mean

**Table 1.** Baseline characteristics of the study population presented as unadjusted means (standard deviation) or percentages

| | Participants | Non-participants* | Adjusted differences† (95% CI)‡ |
|---|---|---|---|
| Number | 1900 | 502 | |
| Age (years) | 64.3 (6.5) | 68.2 (8.3) | 3.9 (3.2; 4.6)\|\| |
| Gender (% female) | 57.0 | 65.0 | 7.8 (2.8; 12.7)\|\| |
| Diabetes mellitus (%) | 4.0 | 7.0 | 2.1 (-3.3; 4.5) |
| Systolic blood pressure (mmHg) | 121.7 (11.8) | 122.7 (11.7) | 0.2 (-1.0; 1.4) |
| Diastolic blood pressure (mmHg) | 68.6 (8.4) | 68.2 (8.8) | 0.6 (-0.3; 1.4) |
| Smoking (%), Current | 24.3 | 28.5 | 8.4 (4.1; 12.8)\|\| |
| Past | 45.1 | 41.5 | -1.6 (-6.4; 3.2) |
| Body mass index (kg/m$^2$) | 25.7 (3.4) | 25.8 (3.7) | 0.0 (-0.4; 0.3) |
| Total serum cholesterol (mmol/l) | 6.60 (1.16) | 6.67 (1.13) | 0.07 (-0.04; 0.19) |
| Serum HDL§ cholesterol (mmol/l) | 1.37 (0.35) | 1.38 (0.38) | 0.00 (-0.03; 0.04) |
| C-reactive protein (mg/l) | 2.40 | 2.99 | 0.55 (0.06; 1.04)\|\| |
| Carotid artery intima media thickness (mm) | 0.74 (0.13) | 0.77 (0.14) | 0.01 (-0.003; 0.003) |
| Retinal arteriolar diameter (µm) | 149.5 (14.0) | 148.6 (14.7) | -0.2 (-1.6; 1.2) |
| Retinal venular diameter (µm) | 223.8 (19.9) | 221.5 (21.5) | -0.4 (-2.5; 1.6) |
| Retinal arteriolar-to-venular ratio | 0.67 (0.05) | 0.67 (0.06) | 1.01 (-0.005; 0.006) |

* Unable or refused at follow-up
† Age and gender adjusted if applicable
‡ 95% CI = 95% confidence interval
§ HDL = high-density lipoprotein
|| Significant ($p < 0.05$)

follow-up of 6.6 years (range: 5.2 to 9.6 years), 808 persons were diagnosed as having incident hypertension at the follow-up examination. Of these incident cases 304 persons were diagnosed with hypertension stage 2.

Table 2 shows that one SD decrease in retinal arteriolar diameters at baseline was associated with a 42% increased risk of incident hypertension. Smaller venular diameters were also related to incident hypertension, although this association was weaker. Each SD decrease in the resulting AVR showed a 29% increase in the risk of incident hypertension. Additional adjustments for other cardiovascular risk factors did not affect the association between retinal vessel diameters and incident hypertension.

Participants in the lowest quartile of arteriolar diameters had compared to those in the highest one a more than twice increased risk of developing hypertension (table 3; OR: 2.22). Table 3 also presents the association between quartiles of retinal venular diameters or AVR and incident hypertension.

**Table 2**. Odds ratios (95% confidence intervals) for incident systemic hypertension (stage 1 or 2) per standard deviation decrease in retinal vessel diameters or arteriolar-to-venular ratio

| | Model I† | Model II‡ |
|---|---|---|
| Retinal arteriolar diameters, per SD* decrease | 1.42 (1.29-1.57) | 1.38 (1.23-1.55) |
| Retinal venular diameters, per SD* decrease | 1.16 (1.05-1.28) | 1.17 (1.04-1.32) |
| Retinal arteriolar-to-venular ratio, per SD* decrease | 1.29 (1.17-1.43) | 1.24 (1.10-1.40) |

* SD = standard deviation

† Age, gender and follow-up time adjusted

‡ Additionally for body mass index, smoking, diabetes mellitus, total and HDL cholesterol, C-reactive protein, intima-media thickness

Stratification on baseline blood pressures revealed that persons, who had an optimal blood pressure, were already at an increased risk of developing hypertension with decreasing arteriolar diameters (table 4). After additional adjustment the relative risk remained unaltered, albeit borderline significant (OR: 1.21; 95% CI: 0.97-1.51).

With the alternative stricter definition for incident hypertension, each SD decrease in retinal arteriolar diameters was still related to an increased risk of hypertension (table 5).

## DISCUSSION

We found that retinal arteriolar narrowing at baseline increased the risk of hypertension in a normotensive elderly population. This association persisted after adjusting for other cardiovascular risk factors and was independent of the baseline blood pressure.

Until recently, prospective data from population-based studies were lacking mainly due to difficulties in reliably assessing arterioles. Of late, several cross-sectional studies, using a semi-automated system to measure retinal vessel diameters, have appeared showing a relationship between smaller retinal arteriolar diameters and increased blood pressures.[9,11,17,18] Also, smaller retinal venular diameters showed a significant, though weaker, association with higher blood pressures.[11,17] Due to the cross-sectional nature of these studies, it was not possible to distinguish cause and consequence. The Atherosclerosis Risk in Communities Study has published data showing a relationship between smaller AVR at baseline and three-years incidence of hypertension (n = 5628; OR per SD decrease: 1.12; 95% CI: 1.03-1.21).[10] The same group confirmed

**Table 3**. Odds ratios (95% confidence intervals) for incident systemic hypertension (stage 1 or 2) in quartiles of retinal vessel diameters or arteriolar-to-venular ratio

| Quartiles [range] | Model I* | Model II† |
|---|---|---|
| Retinal arteriolar diameters (µm) | | |
| 4 [157.7-204.7] | 1.0 (reference) | 1.0 (reference) |
| 3 [148.6-157.6] | 1.10 (0.84-1.44) | 1.00 (0.73-1.36) |
| 2 [140.2-148.5] | 1.31 (1.01-1.71) | 1.15 (0.84-1.58) |
| 1 [95.0-140.1] | 2.22 (1.71-2.89) | 2.15 (1.58-2.93) |
| Retinal venular diameters (µm) | | |
| 4 [236.5-296.4] | 1.0 (reference) | 1.0 (reference) |
| 3 [223.1-236.4] | 1.24 (0.96-1.61) | 1.24 (0.90-1.70) |
| 2 [210.2-223.0] | 1.27 (0.98-1.65) | 1.33 (0.97-1.83) |
| 1 [135.1-210.1] | 1.41 (1.08-1.83) | 1.39 (1.01-1.91) |
| Retinal arteriolar-to-venular ratio | | |
| 4 [0.70-0.94] | 1.0 (reference) | 1.0 (reference) |
| 3 [0.67-0.69] | 1.19 (0.92-1.55) | 1.04 (0.76-1.42) |
| 2 [0.63-0.66] | 1.33 (1.02-1.72) | 1.11 (0.81-1.51) |
| 1 [0.50-0.62] | 1.89 (1.45-2.46) | 1.73 (1.27-2.35) |

* Age, gender and follow-up time adjusted
† Additionally for body mass index, smoking, diabetes mellitus, total and HDL cholesterol, C-reactive protein, intima-media thickness

this finding in data from the Beaver Dam Eye Study (n = 2451; OR per SD decrease: 1.31; 95% CI: 1.18-1.45).[19] The Blue Mountains Eye Study (n = 1319) reported the relation between separate arteriolar diameters and incident hypertension (OR 1st versus 5th quintile: 2.4; 95% CI: 1.6-3.5).[20] In this study, we can confirm the association between smaller arteriolar diameters and incident hypertension. Because smaller venular diameters also increased the risk of hypertension, the magnitude of the association with the AVR was smaller than with the arteriolar diameters separately. Our data thus suggest that in order to determine the risk of hypertension, the arteriolar diameters should be assessed independently. In contrast, in a clinical setting arteriolar diameters are usually gauged against venular ones in order to assess generalized arteriolar narrowing. Our data imply that this approach may underestimate the extent of arteriolar narrowing and thereby the risk of hypertension. Moreover, on

phthalmoscopy one cannot reliably correct for magnification changes due to refractive errors of the eye.

Generalised arteriolar narrowing may be caused by abnormalities in the endothelium, vascular smooth muscle cells or the neuro-muscular junction.[1,2] Local endothelial nitric oxide synthesis (eNOS) in various vascular beds, such as retinal and renal circulation, plays an important role in determining the vessel diameters. Nitric oxide is a potent vasodilator and also prevents smooth muscle cell proliferation.[21] In normotensive persons administration of N-mono-methyl-L-arginine, an inhibitor of eNOS, resulted in elevated systemic blood pressure and decreased retinal blood flow.[22] In contrast, no change occurred in hypertensive patients, suggesting that eNOS might already be disturbed. Impaired local production of other vaso-active agents (angiotensin II, endothelin-1) could also contribute to an altered peripheral vascular resistance. Another mechanism could be hypersensitivity of smooth muscle cells to these vaso-active agents.[23] Hypertensive patients manifest greater vasoconstrictor responses to infused norepinephrine than normotensive persons.[24] Moreover, vasoconstrictor responsiveness is increased in normotensive offspring of hypertensive

**Table 4**. Odds ratios (95% confidence intervals) for incident systemic hypertension per standard deviation decrease in retinal vessel diameters or arteriolar-to-venular ratio stratified on baseline blood pressure

| | Baseline blood pressure | |
|---|---|---|
| | **Optimal* (189/739)‡** | **Normal to borderline† (619/1161)‡** |
| Retinal arteriolar diameters, per SD§ decrease | | |
| Model I\|\| | 1.29 (1.08-1.56) | 1.37 (1.22-1.56) |
| Model II# | 1.21 (0.97-1.51) | 1.38 (1.20-1.59) |
| Retinal venular diameters, per SD§ decrease | | |
| Model I\|\| | 1.06 (0.88-1.27) | 1.17 (1.03-1.32) |
| Model II# | 1.02 (0.81-1.28) | 1.21 (1.05-1.40) |
| Retinal arteriolar-to-venular ratio, per SD§ decrease | | |
| Model I\|\| | 1.25 (1.04-1.50) | 1.24 (1.09-1.41) |
| Model II# | 1.22 (0.98-1.53) | 1.21 (1.05-1.39) |

* Defined as systolic blood pressure < 120 mmHg and diastolic blood pressure < 80 mmHg
† Defined as systolic blood pressure of 120 to 139 mmHg or diastolic blood pressure of 80 to 89 mmHg
‡ Number of incident hypertension cases / Total number of persons within stratum
§ SD = standard deviation
|| Adjusted for age, gender and follow-up time
# Additionally for body mass index, smoking, diabetes mellitus, total and HDL cholesterol, C-reactive protein, intima-media thickness

**Table 5**. Odds ratios (95% confidence intervals) for incident systemic hypertension stage 2 per standard deviation decrease in retinal vessel diameters or arteriolar-to-venular ratios

| | Model I† | Model II‡ |
|---|---|---|
| Retinal arteriolar diameters, per SD* decrease | 1.60 (1.39-1.86) | 1.62 (1.36-1.92) |
| Retinal venular diameters, per SD* decrease | 1.18 (1.03-1.34) | 1.22 (1.03-1.44) |
| Retinal arteriolar-to-venular ratio, per SD* decrease | 1.43 (1.24-1.66) | 1.40 (1.17-1.66) |

* SD = standard deviation

† Age, gender and follow-up time adjusted

‡ Additionally for body mass index, smoking, diabetes mellitus, total and HDL cholesterol, C-reactive protein, intima-media thickness

parents suggesting that hypersensitivity of these cells may be primary in origin.[24] Finally, disturbed sympathetic activity in arterioles such as greater release of neurotransmitters,[25] altered neuronal neurotransmitter reuptake,[26] or sympathetic neuro-modulation by angiotensin-II[27] may contribute to the development of hypertension. Based on the retinal intra-luminal diameters we measured we cannot, however, distinguish which of the above-mentioned mechanisms may lead to arteriolar narrowing.[10,19,20]

Previously, we showed that smaller arteriolar diameters were also cross-sectionally related to hypertension.[11] It could be that the associations we described were explained by the fact that some of the incident cases already had hypertension at baseline, but were misclassified as normotensive due to large within-subject variability for blood pressures. In our study, the SD of within-subject variability for blood pressure was 10.8 mmHg. Therefore, if there was misclassification, this most probably might have affected those who were normotensive at baseline and had hypertension stage 1 at follow-up. Hence, we excluded these persons and repeated the analyses by taking only those who developed hypertension stage 2 at follow-up. The association between baseline retinal vessel diameters and incident hypertension was still present.

Another limitation is the number of non-participants at follow-up. Although non-participants were older, more often women, more often current smokers and had a higher CRP compared to participants, there were no major differences in other cardiovascular risk factors between these groups. Especially, baseline blood pressures and retinal vessel diameters did not show any differences, suggesting that there was no selective non-response. Strengths of our study are the prospective population-based design with a long follow-up, identical examination procedures at baseline and follow-up

for blood pressure measurement, and detailed and standardized evaluation of retinal arteriolar diameters.

In conclusion, data from this prospective population-based study in an elderly population provide further evidence that generalised retinal arteriolar narrowing may precede the development of systemic hypertension by seven years.

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# Chapter 5.2

# Retinal Vessel Diameters and Risk of Diabetes Mellitus: The Rotterdam Study


## ABSTRACT

*The association between a smaller retinal arteriolar-to-venular diameter ratio (AVR) and incident diabetes mellitus may be due to arteriolar narrowing or venular dilatation. We investigated if smaller arteriolar or larger venular diameters were associated with incident impaired fasting glucose or diabetes in a population-based cohort (≥ 55 years). Retinal vessel diameters at baseline (1990-1993) were measured on digitized images of 2309 persons, who had a normal glucose tolerance test (post-load glucose < 7.8 mmol/l). At the follow-up examination (1997-1999), incident impaired fasting glucose was defined as 6.1-7.0 mmol/l, and diabetes as ≥ 7.0 mmol/l and/or use of anti-diabetic medication. Adjusted for age, gender and follow-up time, odds ratios (OR) per standard deviation (SD) increase in venular diameters were 1.13 (95% confidence interval: 1.00-1.29) for impaired fasting glucose and 1.09 (0.90-1.33) for diabetes. Odds ratios per SD decrease in arteriolar diameters were 1.12 (0.98-1.27) and 1.08 (0.89-1.31), and per SD decrease in AVR were 1.29 (1.13-1.46) and 1.19 (0.98-1.45). After adjustment for cardiovascular risk factors, the associations were unaltered for venular diameters and disappeared for arteriolar diameters. The associations of venular dilatation with impaired fasting glucose (OR: 1.23; 1.02-1.47) or diabetes (OR: 1.18; 0.89-1.56) were stronger in the younger participants. In conclusion, the risk of impaired fasting glucose and diabetes mellitus with AVR was largely explained by venular dilatation rather than arteriolar narrowing warranting more focus on the causes of this dilatation.*

Submitted

Epidemiological studies have shown that high body mass index, parental history of diabetes and impaired glucose tolerance are some of the factors involved in the development of diabetes mellitus (1; 2). Microvascular changes such as impaired microvascular reactivity and disturbed blood flow in microvessels have also been hypothesized to contribute to the pathogenesis of diabetes mellitus (3; 4). Several cross-sectional studies have shown these abnormalities not only in persons with diabetes mellitus (3), but also in those at high risk of developing diabetes mellitus, including persons with an impaired glucose tolerance, or first-degree relatives of diabetes patients (4).

Until recently, prospective population-based data regarding the role of microvascular abnormalities in diabetes were lacking due to difficulties in non-invasively assessing the microcirculation in vivo. A semi-automated system was developed to measure retinal vessel diameters (5). It was suggested that the retinal arteriolar-to-venular ratio (AVR) thus obtained was a marker of generalized arteriolar narrowing and that a smaller AVR was related to incident diabetes mellitus (6). Due to the nature of ratio measures like this one, it is difficult to judge whether this relation is due to retinal arteriolar narrowing or venular dilatation. Studying the arteriolar and venular diameters

separately may be important for elucidating the microvascular complications of diabetes mellitus, such as retinal venular dilatation that has been reported to be present already in the pre-diabetic stage of hyperglycemia (7-9).

Therefore, we investigated in the population-based Rotterdam Study whether the AVR was associated with an increased risk of impaired fasting glucose or diabetes mellitus and whether this was explained by retinal arteriolar narrowing or venular dilatation.

## RESEARCH DESIGN AND METHODS

### Study population

The present study was performed as part of the Rotterdam Study, a prospective population-based cohort study (10). A total of 7983 inhabitants of a district of Rotterdam, aged 55 years and older, agreed to participate in the study (response rate of 78%). Because the ophthalmic part became operational after the screening had already started, a smaller number (n = 6780) underwent the ophthalmic examination (11). The study was conducted according to the tenets of the Declaration of Helsinki, and the medical ethics committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline interviews and examinations took place from 1990 to mid-1993, and the follow-up screening occurred between 1997 and 1999.

### Retinal vessel diameter measurements at baseline

Retinal vessel diameters were measured on fundus color transparencies. Fundus color transparencies covering a 20° field, centered on the optic disc, were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan) (12). For each person the image of one eye with the best quality was chosen and analyzed with a semi-automated system (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison) by four trained graders masked for the endpoints (11). We used the improved Parr-Hubbard formula to compute the summary vessel measures (13), and additionally adjusted for the differences in magnification due to the refractive power of the eye (14). Sum values were calculated for the arteriolar and venular blood column diameters (in μm), the AVR being the ratio of these. In a random sub-sample of 100 participants we found no differences between the right and left eyes for the arteriolar and venular diameters.

**Ascertainment of incident cases of impaired fasting glucose and diabetes mellitus**

At baseline a non-fasting blood sample was drawn by venipuncture, and after that, an oral glucose tolerance test was performed in participants who were not on anti-diabetic medication. These participants received a drink containing 75 g of glucose (15). Two hours later, a second blood sample was obtained. Serum was separated by centrifugation and quickly frozen in liquid nitrogen. Glucose levels were measured by the glucose hexokinase method (15).

At the follow-up examination, the oral glucose tolerance test was not performed; instead blood samples were drawn at the research center after overnight fasting (8-14 hours). Definitions for incident cases of impaired fasting glucose and diabetes mellitus were adapted from the American Diabetes Association guidelines (16; 17). For the current study, only persons who had a normal oral glucose tolerance test (post-load glucose value < 7.8 mmol/l) and did not use anti-diabetic medication at baseline were included. Incident impaired fasting glucose was defined as having a fasting glucose value between 6.1 to 7.0 mmol/l, and incident diabetes mellitus as fasting blood glucose value ≥ 7.0 mmol/l at the follow-up examination. Also, persons who started using anti-diabetic medication during follow-up were classified as having incident diabetes mellitus.

**Assessment of confounders**

Blood pressure was measured in sitting position at the right brachial artery with a random-zero sphygmomanometer. In the analyses we used the average of two measurements taken at one occasion. Information on smoking habits was derived from the baseline interview. Smoking was categorized as never, former or current. Body mass index was computed as weight divided by height squared ($kg/m^2$). Non-fasting serum total cholesterol was determined by an enzymatic procedure and high-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction (18). Serum levels of C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Immage® high sensitive CRP, Beckman Coulter, USA). Atherosclerotic plaques, assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides, were defined as focal thickening of the vessel wall (of at least 1.5 times the average intima-media thickness) relative to adjacent segments with or without calcified components. The carotid plaque score (range:0-6) reflected the number of these locations with plaques (19).

**Study sample**

Of the 6780 participants in the ophthalmic part of the baseline study, 5674 persons had gradable fundus transparencies for retinal vessel measurements. Data on glucose tolerance test and anti-diabetic medication were available for 4972 persons. Of these, 3699 participants had a normal glucose tolerance test and did not use anti-diabetic medication at baseline and thus formed the cohort at risk of impaired fasting glucose or diabetes mellitus. During follow-up 443 participants died, a further 947 refused or were unable to participate at the follow-up examination leaving 2309 persons for the current analyses.

**Statistical analysis**

Analysis of covariance was used to compare baseline characteristics of participants to non-participants at follow-up. Arteriolar and venular diameters, and AVR were analyzed both in quartiles and linearly (per standard deviation (SD) difference) with logistic regression models. Odds ratios (OR) and 95% confidence interval (CI) were calculated for incident impaired fasting glucose and diabetes mellitus. Participants who developed impaired fasting glucose during follow-up were excluded from analyses where diabetes was the outcome, and vice versa. For additional adjustments we included those cardiovascular risk factors that we previously showed to be associated with retinal vessel diameters (11), and that also have been implicated in the pathogenesis of diabetes mellitus. We adjusted for age, gender, follow-up time, and additionally for body mass index, diastolic and systolic blood pressure, smoking, total and HDL cholesterol levels, CRP and carotid artery plaque score. We repeated the analyses after stratifying on age. The cut-off value of 70 years was chosen to secure enough cases in both strata. All analyses were performed with SPSS Windows version 11.0 (SPSS Inc., Chicago, Illinois).

**RESULTS**

Table 1 shows that non-participants were on average older, more often women and smokers, and had higher blood pressures and more plaques in the carotid arteries. Persons who died during follow-up had a worse cardiovascular risk profile compared to participants. After a mean follow-up time of 6.4 years (range: 5.2-9.5), 305 participants developed impaired fasting glucose and 118 diabetes mellitus. In persons who developed impaired fasting glucose the mean arteriolar diameter at baseline was 146.3μm (SD: 13.5), venular diameter 224.8μm (SD: 20.6) and AVR 0.65 (SD: 0.05), whereas in those

**Table 1.** Baseline Characteristics Presented as Unadjusted Means (Standard Deviation) or Percentages

| | Participants | Non-participants* | Adjusted differences†‡ (95% CI)§ | Died\|\| | Adjusted differences†# (95% CI)§ |
|---|---|---|---|---|---|
| Number | 2309 | 947 | | 443 | |
| Age (years) | 65.0 (6.5) | 68.7 (8.1) | 3.7 (3.1; 4.2)†† | 74.4 (8.4) | 9.4 (8.7; 10.1)†† |
| Gender (% female) | 57.0 | 62.0 | 4.4 (0.6; 8.2)†† | 47.0 | -12.3 (-17.7; -6.8)†† |
| Systolic blood pressure (mmHg) | 133.7 (20.0) | 139.4 (22.9) | 3.2 (1.6; 4.8)†† | 143.8 (23.5) | 3.3 (1.0; 5.6)†† |
| Diastolic blood pressure (mmHg) | 73.3 (10.8) | 74.2 (11.4) | 1.3 (0.4; 2.2)†† | 73.6 (12.3) | 1.1 (-0.2; 2.3) |
| Smoking (%), Current | 21.8 | 28.2 | 10.2 (7.0; 13.5)†† | 27.9 | 13.9 (9.2; 18.6)†† |
| Body mass index (kg/m$^2$) | 26.2 (3.4) | 26.3 (3.7) | 0.0 (-0.3; 0.3) | 25.8 (3.7) | -0.6 (-0.9; -0.2)†† |
| Total serum cholesterol (mmol/l) | 6.66 (1.16) | 6.68 (1.17) | 0.02 (-0.07; 0.11) | 6.30 (1.28) | -0.22 (-0.34; -0.09)†† |
| Serum HDL** cholesterol (mmol/l) | 1.38 (0.35) | 1.39 (0.36) | 0.01 (-0.02; 0.03) | 1.31 (0.36) | -0.03 (-0.06; 0.01) |
| C-reactive protein (mg/l) | 2.42 | 2.74 | 0.33 (-0.06; 0.72) | 5.01 | 2.44 (1.88; 3.00)†† |
| Number of carotid artery plaques ≥ 4 (%) | 11.0 | 17.1 | 3.9 (1.0; 6.8)†† | 24.0 | 5.6 (1.5; 9.8)†† |
| Retinal arteriolar-to-venular ratio | 0.67 (0.06) | 0.66 (0.06) | -0.002 (-0.006; 0.003) | 0.66 (0.06) | -0.009 (-0.016; -0.003)†† |
| Retinal arteriolar diameter (μm) | 147.7 (14.1) | 146.4 (14.4) | -0.4 (-1.5; 0.7) | 145.0 (15.0) | -0.5 (-2.1; 1.1) |
| Retinal venular diameter (μm) | 222.8 (19.9) | 220.9 (20.8) | 0.1 (-1.5; 1.6) | 221.1 (20.5) | 2.5 (0.3; 4.7)†† |

* Unable or refused at follow-up
† Age and gender adjusted if applicable
‡ Non-participants versus Participants
§ 95% CI = 95% confidence interval
|| Persons who died before the follow-up examination
# Deceased persons versus Participants
** HDL = high-density lipoprotein
†† Significant ($p < 0.05$)

**Table 2.** Odds ratios* of incident Impaired Fasting Glucose or incident Diabetes Mellitus per quartile of baseline retinal vessel diameters or arteriolar-to-venular ratio

| Quartiles [range] | Impaired Fasting Glucose (n = 305) | Diabetes Mellitus (n = 118) |
|---|---|---|
| Arteriolar diameter (μm) | | |
| 4. [156.4-204.7] | 1.0 (reference) | 1.0 (reference) |
| 3. [147.1-156.3] | 1.04 (0.73-1.49) | 1.42 (0.82-2.46) |
| 2. [137.8-147.0] | 1.29 (0.92-1.82) | 1.58 (0.92-2.72) |
| 1. [95.0-137.7] | 1.17 (0.82-1.66) | 1.29 (0.73-2.28) |
| Venular diameter (μm) | | |
| 1. [135.1-209.0] | 1.0 (reference) | 1.0 (reference) |
| 2. [208.9-222.1] | 1.33 (0.94-1.90) | 1.24 (0.73-2.12) |
| 3. [222.0-235.4] | 1.20 (0.84-1.72) | 0.91 (0.52-1.62) |
| 4. [235.3-313.6] | 1.46 (1.02-2.08) | 1.41 (0.83-2.38) |
| Arteriolar-to-venular ratio | | |
| 4. [0.70-0.87] | 1.0 (reference) | 1.0 (reference) |
| 3. [0.66-0.69] | 1.37 (0.94-2.00) | 1.33 (0.76-2.33) |
| 2. [0.63-0.65] | 1.64 (1.13-2.36) | 1.30 (0.73-2.30) |
| 1. [0.49-0.62] | 1.93 (1.35-2.77) | 1.80 (1.05-3.08) |

***** Odds ratios (95% confidence interval) were adjusted for age, gender and follow-up time

who developed diabetes these values were 146.4μm (SD: 13.5), 224.1μm (SD: 19.8) and 0.66 (SD: 0.06). In the remaining participants the mean arteriolar diameter was 147.9μm (SD: 14.2), venular diameter 222.4 (SD: 19.7) and AVR 0.67 (SD: 0.06).

The lowest quartile of arteriolar diameters compared to the highest one was neither related to an increased risk of impaired fasting glucose, nor to the risk of diabetes mellitus (table 2). Comparing the highest quartile of venular diameters to the lowest one showed a 46% increased risk of impaired fasting glucose. There was no consistent trend between quartiles of venular diameters and the risk of diabetes mellitus. Finally, the lowest quartile of AVR compared to the highest one gave a significantly increased risk of 93% for impaired fasting glucose, and of 80% for diabetes mellitus.

Table 3 shows that each SD decrease in arteriolar diameters increased the risk of impaired fasting glucose by 12%, whereas one SD increase in venular diameters resulted in a 13% increased risk. The corresponding non-

**Table 3.** Odds ratios* of incident Impaired Fasting Glucose or incident Diabetes Mellitus per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio

| | Impaired Fasting Glucose (n = 305) | Diabetes Mellitus (n = 118) |
|---|---|---|
| Arteriolar narrowing† | 1.12 (0.98-1.27) | 1.08 (0.89-1.31) |
| Venular dilatation‡ | 1.13 (1.00-1.29) | 1.09 (0.90-1.33) |
| Arteriolar-to-venular ratio§ | 1.29 (1.13-1.46) | 1.19 (0.98-1.45) |

* Odds ratios (95% confidence interval) were adjusted for age, gender and follow-up time
† Per standard deviation decrease in arteriolar diameter
‡ Per standard deviation increase in venular diameter
§ Per standard deviation decrease in arteriolar-to-venular ratio

significant increases in risk for diabetes mellitus were 8% and 9%. A decrease in the resulting AVR at baseline was associated with significantly increased risk of impaired fasting glucose and borderline with diabetes mellitus. Each SD decrease in AVR the risk of impaired fasting glucose increased by 29% and of diabetes mellitus by 19%.

Table 4 shows that after adjusting for other known cardiovascular risk factors, the associations of smaller arteriolar diameters with impaired fasting glucose and diabetes mellitus completely disappeared. The strength of the association between larger venular diameters and impaired fasting glucose remained the same, albeit borderline significant. Even for diabetes the non-significant OR remained the same.

Stratification on age revealed that the associations of larger venular diameters with impaired fasting glucose or diabetes mellitus were stronger in younger and absent in the older participants (table 5).

## DISCUSSION

Our data provide evidence that retinal venular dilatation rather than arteriolar narrowing explained the associations of smaller AVR with impaired fasting glucose and diabetes mellitus. With respect to venular dilatation, the strength of the associations was more pronounced in the younger participants.

It has been suggested that venular dilatation is one of the early changes in the microcirculation of diabetic patients (20). Increase in venular diameters is thought to result from retinal hypoxia and lactate accumulation (20; 21). Successful treatment of proliferative diabetic retinopathy with retinal

**Table 4.** Multivariable adjusted odds ratios* of incident Impaired Fasting Glucose or incident Diabetes Mellitus per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio

| | **Impaired Fasting Glucose (n = 305)** | **Diabetes Mellitus (n = 118)** |
|---|---|---|
| Arteriolar narrowing† | 0.99 (0.86-1.15) | 1.02 (0.82-1.27) |
| Venular dilatation‡ | 1.15 (0.99-1.34) | 1.08 (0.86-1.36) |
| Arteriolar-to-venular ratio§ | 1.14 (0.98-1.32) | 1.09 (0.87-1.36) |

* Odds ratios (95% confidence interval) were adjusted for age, gender, follow-up time, diastolic and systolic blood pressure, smoking, body mass index, serum total and HDL cholesterol, C-reactive protein and carotid artery plaque score
† Per standard deviation decrease in arteriolar diameter
‡ Per standard deviation increase in venular diameter
§ Per standard deviation decrease in arteriolar-to-venular ratio

photocoagulation has been shown to be accompanied by a reduction in venular diameters, possibly due to less retinal hypoxia (21). In normoglycemic individuals, it has been observed that administration of intravenous dextrose resulted in retinal venular dilatation (7). In a case-control study, 266 patients with juvenile diabetes mellitus had significantly larger venular diameters (396.5μm) compared to 129 controls (368.0μm) (8). In a population-based cohort study, which consisted of 996 diabetic patients, larger venualr diameters were associated with 4-years progression of retinopathy (relative risk [RR] 4$^{th}$ versus 1$^{st}$ quartile: 2.3; 95% CI: 1.4-4.0) and with 14-year incidence of proliferative retinopathy (RR: 4.3; 95% CI: 1.5-12.2) (21). Vasodilatation of venules and to a lesser extent arterioles has also been described in other vascular beds, including the renal and cerebral circulation (22). However, the actual mechanisms underlying venular dilatation remain unclear.

There are several explanations for the fact that the associations were stronger and more significant for impaired fasting glucose than for diabetes mellitus. We had three times more cases with impaired fasting glucose than with diabetes. There might be an under representation of diabetic cases in our study population, because non-participants were at baseline significantly older, had higher blood pressures, were more often smokers, and had more carotid artery plaques. As these risk factors also increase the risk of diabetes mellitus, the non-participants would have more often developed diabetes mellitus. Furthermore, those who died during follow-up had an even worse cardiovascular risk profile, including significantly larger venular diameters after adjustment for age and gender. Because those who develop diabetes

**Table 5.** Multivariable adjusted odds ratios* of incident Impaired Fasting Glucose or incident Diabetes Mellitus per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio stratified on age

| | Impaired Fasting Glucose (cases) | Diabetes Mellitus (cases) |
|---|---|---|
| Age < 70 | (231) | (88) |
| Arteriolar narrowing† | 1.00 (0.85-1.19) | 0.98 (0.76-1.28) |
| Venular dilatation‡ | 1.23 (1.02-1.47) | 1.18 (0.89-1.56) |
| Arteriolar-to-venular ratio§ | 1.21 (1.01-1.45) | 1.13 (0.86-1.47) |
| Age ≥ 70 | (74) | (30) |
| Arteriolar narrowing† | 0.93 (0.68-1.26) | 1.08 (0.71-1.65) |
| Venular dilatation‡ | 1.02 (0.76-1.37) | 0.91 (0.60-1.37) |
| Arteriolar-to-venular ratio§ | 0.95 (0.70-1.29) | 1.00 (0.67-1.49) |

* Odds ratios (95% confidence interval) were adjusted for age, gender, follow-up time, diastolic and systolic blood pressure, smoking, body mass index, serum total and HDL cholesterol, C-reactive protein and carotid artery plaque score
† Per standard deviation decrease in arteriolar diameter
‡ Per standard deviation increase in venular diameter
§ Per standard deviation decrease in arteriolar-to-venular ratio

are more likely to die than those who have normal glucose, it is possible that there is selective non-participation of persons who had larger venular diameters and developed diabetes. Another limitation is the use of different methods to diagnose diabetes mellitus at baseline and follow-up examinations. Because of its inconvenience to patients, the oral glucose tolerance test was not performed at the follow-up examination. Instead, it was decided to use glucose levels after overnight fast. It has been reported that diagnosing diabetes mellitus with fasting glucose levels would result in fewer cases than with oral glucose tolerance test, especially in the elderly (23; 24). This misclassification could further have shifted the point estimate towards the null-value. Given the fact that impaired fasting glucose can be considered a pre-diabetic stage of hyperglycemia, it seems justified to assume that potential risk factors of impaired fasting glucose also increase the risk of diabetes mellitus (23).

The different associations might also be explained by the decreased vasodilatory capacity at baseline in patients who developed diabetes due to an older age distribution or co-morbidity such as hypertension compared to those who developed impaired fasting glucose. However, in our population both mean age and blood pressure at baseline were not significantly different between the two groups. What we did find is that although all participants

had a normal oral glucose tolerance test at baseline, persons who developed incident diabetes mellitus (6.21 mmol/l) already had a significantly higher post-load glucose level at baseline than those who developed impaired fasting glucose (5.98 mmol/l). A higher glucose level could result in an accelerated formation of advanced glycation end products, which can accumulate in various vascular beds including retina leading to vascular wall stiffness and decreased endothelium-dependent vasodilatation (25).

Prospective data from Atherosclerosis Risk in Communities (ARIC) Study showed that each SD decrease in the AVR was related to a 26% increased risk of diabetes mellitus (6). However, other retinal arteriolar abnormalities such as arterio-venous nicking (OR: 0.94; 95% CI: 0.65-1.35) and focal arteriolar narrowing (OR: 1.12; 95% CI: 0.78-1.59) were not related to diabetes mellitus (6). Based on these data the authors concluded that generalized arteriolar narrowing may play a role in its initial development (6). In our study, we confirmed the findings with respect to the AVR. However, our data suggest that venular dilatation might be more relevant for explaining these findings. Furthermore, the associations with smaller arteriolar diameters disappeared after additional adjustments. We did not have information on other retinal arteriolar signs (focal arteriolar narrowing, arterio-venous nicking). With respect to venular diameters, we have previously shown that larger diameters were related to markers of atherosclerosis, higher levels of inflammation and smoking (11). Because these factors also give an increased risk of diabetes mellitus, these factors could underlie the associations we have described. Although non-significant, the associations with venular dilatation remained unaltered after adjustment for these factors (table 4). Given that the ARIC, which found stronger associations between AVR and diabetes (6), studied younger people, we decided to stratify on age. Our data also revealed stronger associations in the younger age category. In the older persons venular dilatation was not related to impaired fasting glucose or diabetes probably due to decreased vasodilatory capacity in these persons.

In conclusion, the association of AVR with impaired fasting glucose and diabetes mellitus seemed to be largely explained by venular dilatation. These data support the view that venular changes are present already in the early stages in the development of diabetes mellitus. In a clinical setting, venular changes such as dilatation should also be assessed in order to explore the full extent of microvascular abnormalities in diabetes. Further research needs to be conducted to elucidate the actual mechanisms that lead to these alterations.

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# Chapter 5.3

# Retinal Vessel Diameters and All-Cause Mortality: The Rotterdam Study


## ABSTRACT

***Background*** *– Retinal vessels are used to assess systemic vascular damage. A smaller arteriolar-to-venular diameter ratio is associated with an increased risk of cardiovascular diseases. With respect to mortality, data were inconsistent. The inconsistent associations between a lower ratio and mortality might be caused by arteriolar narrowing or venular dilatation. We examined whether smaller arteriolar, larger venular diameters or both predict 10-year all-cause mortality.*

***Methods*** *– This study was based on the prospective population-based Rotterdam Study and included 5540 participants of 55 years or over. At baseline (1990-1993), arteriolar and venular diameters, corrected for magnification changes in case of ametropia, were measured on digitized images. Participants were followed up until date of death, date when lost to follow-up, or until January 1, 2003, whichever came first.*

***Results*** *– After a mean follow-up of 9.9 years, 1762 participants died. Venular diameters were associated with an increased risk of mortality (age- and gender-adjusted hazard ratio (HR) per standard deviation (SD) increase: 1.07; 95% confidence interval (CI): 1.02-1.12), whereas arteriolar diameters were not (HR per SD decrease: 1.00; 95% CI: 0.95-1.05). The arteriolar-to-venular ratio was also related to mortality (HR per SD decrease: 1.07; 95% CI: 1.02-1.12). Stratification on age showed that the association between venular diameters and mortality was more prominent in participants below 70 years (HR per SD increase: 1.18; 95% CI: 1.09-1.29). Additional adjustments for cardiovascular risk factors did not alter these results.*

***Conclusions*** *– Our data show that retinal venular dilatation predicts all-cause mortality independently of other cardiovascular risk factors, especially in younger participants.*


To be submitted

Since the late 19th century, retinal vessel signs (e.g. generalized arteriolar narrowing and arterio-venous nicking) were recognized as markers of systemic vascular damage, and were used to predict life prognosis.[1,2] Keith, Wagener and Barker were among the first to relate these fundus signs to survival of patients having systemic hypertension.[3] The 3-year survival of patients with grade I (mild arteriolar narrowing) was 70%, compared to 6% in those with grade IV (marked arteriolar narrowing, exudates and papilledema). In the following decades, other studies showed similar findings.[4-6] Inferences from these studies to general populations were limited because most of this pioneering work was performed in clinic-based hypertensive patients in a period with limited treatment possibilities, with small sample sizes and inadequate control for confounders.[7] Also, ophthalmoscopic detection of these signs, especially generalized arteriolar narrowing, was shown to be unreliable.[1,8,9]

Of late, a semi-automated system was developed to measure retinal vessel diameters more accurately.[8,10] A smaller arteriolar-to-venular diameter ratio (AVR) thus acquired was associated with increased risk of cardiovascular diseases.[11,12] With respect to mortality, data were inconsistent.[13,14] In a case-control study nested in the Beaver Dam Eye Study, a weak association was

reported between a smaller AVR and cardiovascular mortality.[14] When the entire cohort was analyzed this finding could not be confirmed.[13]

In view of these inconsistencies, we examined the relationship between retinal vessel diameters and all-cause mortality. Previously, we showed that smaller arterioles were related to higher blood pressures and larger venules to markers of atherosclerosis and inflammation. Because a lower AVR could be due to both arteriolar narrowing or venualr dilatation, it is difficult to pinpoint its pathophysiological basis.[15] Therefore, we separately measured arteriolar and venular diameters hypothesizing, that smaller arterioles or larger venules diameters might predict the risk of all-cause mortality.

## PARTICIPANTS AND METHODS

### Population

The present study was performed as part of the Rotterdam Study, a prospective population-based, cohort study on chronic diseases in the elderly.[16] All inhabitants of a district of the city of Rotterdam aged 55 years and over were invited in random order to the study, and 7983 actually participated (overall response 78%). Because the ophthalmic part became only operational after the study had started, a total of 6780 participants underwent the ophthalmic examination. The study was conducted according to the Declaration of Helsinki, and the Medical Ethics Committee of the Erasmus Medical Center approved the study protocol. A written informed consent was obtained from all participants. Baseline home interviews and examinations were performed from 1990 to mid-1993.

### Retinal vessel measurements

Participants underwent a full eye examination at baseline including simultaneous stereoscopic fundus color photography of the optic disc ($20^0$ field, Topcon Optical Company, Tokyo, Japan) after pharmacological mydriasis. The transparencies from both eyes were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan), and for each participant the digitized image of one eye with the best quality was analyzed with the Retinal Vessel Measurement System (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison).[10] For each participant one summary value was calculated for the arteriolar diameters (in μm) of the blood column and one for the venular diameters after correction for differences in magnification due to refractive status of the eye; the AVR was defined as the ratio of arteriolar to venular

diameters.[17-21]

In a random sub-sample of 100 participants we found no statistically significant differences between the right and left eyes for the arteriolar and venular diameters. Four trained graders performed these measurements masked for participant characteristics. Pearson's correlation coefficients for inter-grader agreement were for arteriolar diameters 0.67-0.80, for venular diameters 0.91-0.94 and for AVR 0.75-0.84. For intra-grader agreement the corresponding figures were 0.69-0.88, 0.90-0.95 and 0.72-0.90.

**All-cause mortality**

From March 1990 until January 1, 2003, information on vital status of the entire cohort was obtained from the municipal health services in Rotterdam on a biweekly basis. Also, the general practitioners in the study area reported deaths on a continuous basis. Specially trained study personnel verified reported deaths by checking the medical records. Participants were followed up from the date of entry until the date of death, the date they were lost to follow-up, or January 1, 2003, whichever came first. Data on vital status was nearly complete (99.9%).

**Assessment of confounders**

Baseline blood pressure was measured in sitting position at the right brachial artery with a random-zero sphygmomanometer. In the analyses we used the average of two measurements taken at one occasion. Body mass index (BMI) was computed as weight divided by height squared. Non-fasting serum total and HDL cholesterol concentrations were determined by an automated enzymatic procedure.[22] Diabetes mellitus was considered present if participants reported use of antidiabetic medication or when random or post-load serum glucose level was greater than 11 mmol/l. Serum levels of C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Immage® high sensitive CRP, Beckman Coulter, USA). Atherosclerotic plaques, assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides, were defined as focal thickening of the vessel wall (of at least 1.5 times the average intima-media thickness) relative to adjacent segments with or without calcified components. The carotid artery plaque score (range: 0 to 6) reflected the number of these locations with plaques.[23] Information on smoking (categorized as current, former or never) and antihypertensive medication use was obtained during the home interview by a computerized questionnaire.

**Statistical analyses**

Analysis of covariance, adjusted for age and gender, was used to assess differences in baseline characteristics between persons who died during follow-up to those who were still alive at the end of the follow-up period. Associations of baseline retinal vessel diameters or AVR with mortality were analyzed using Cox proportional hazards models. Hazard ratios (HR) with corresponding 95% confidence intervals (CI) for mortality were calculated by analyzing retinal vessel diameters both linearly per standard deviation (SD) and in quartiles. Initial adjustments were made for age and gender; we additionally adjusted for baseline levels of smoking, diabetes mellitus, carotid artery plaque score, body mass index, serum total and high-density lipoprotein cholesterol, C-reactive protein, systolic and diastolic blood pressure, and anti-hypertensive medication. All analyses were performed using SPSS (Windows version 11.0; SPSS inc., Chicago, Illinois).

## RESULTS

Table 1 presents the baseline characteristics of participants, comparing those who died during follow-up with those still alive as of January 1, 2003. Persons who died had a significantly worse cardiovascular risk profile compared to the persons who were still alive. During a total of 56,229 persons-years (mean follow-up time per person: 9.9 (SD: 3.1)) 1762 persons died. The mean arteriolar diameter at baseline was 146.9μm (range: 92.2-235.7; SD: 14.4), venular diameter 222.0μm (range: 135.1-313.6; SD: 20.9) and AVR 0.66 (0.48-1.02; SD: 0.06).

Table 2 shows that with each SD increase in venular diameters the risk of mortality increased significantly by 7%. Smaller arteriolar diameters were not associated with an increased risk of mortality. The resulting AVR revealed that each SD decrease was also associated with a 7% increased risk of mortality.

Table 3 presents the analyses after categorizing retinal vessel diameters into quartiles. Participants in the highest quartile of venular diameters had a 19% increased risk of mortality compared to those in the lowest quartile. Quartiles of arteriolar diameters were not related to the risk of mortality. Participants in the lowest quartile of AVR had a 1.2 times higher risk of mortality compared with those in the highest quartile.

In table 4 the HRs for the associations between retinal vessel diameters and mortality are presented after additional adjustment for other cardiovascular risk factors. The strength of the associations hardly changed

**Table 1**. Baseline characteristics

| | Alive* | Dead† | Adjusted differences‡ (95% CI)§ |
|---|---|---|---|
| Number (n) | 3912 | 1762 | |
| Age (years) | 65.4 (6.7) | 73.7 (8.2) | 8.2 (7.8; 8.6)\|\| |
| Gender (%, female) | 62 | 52 | -17.4 (-20.5; -14.3)\|\| |
| Institutionalized (%) | 1 | 10 | 3.3 (2.2; 4.4)\|\| |
| Diabetes mellitus (%) | 6 | 17 | 7.5 (5.7; 9.4)\|\| |
| Smoking (%, current) | 21.7 | 27.9 | 14.1 (11.4; 16.7)\|\| |
| Body mass index (kg/m$^2$) | 26.4 (3.6) | 26.1 (3.8) | -0.29 (-0.53; -0.06)\|\| |
| Systolic blood pressure (mmHg) | 136.1 (21.0) | 144.1 (23.5) | 2.95 (1.57; 4.34)\|\| |
| Diastolic blood pressure (mmHg) | 73.8 (10.9) | 73.4 (12.3) | 0.5 (-0.2; 1.3) |
| Number of carotid artery plaques ≥ 4 (%) | 12 | 26 | 7.7 (5.1; 10.2)\|\| |
| Serum total cholesterol (mmol/l) | 6.72 (1.18) | 6.46 (1.24) | -0.10 (-0.18; -0.02)\|\| |
| Serum HDL# cholesterol (mmol/l) | 1.37 (0.35) | 1.31 (0.38) | -0.02 (-0.04; 0.00) |
| C-reactive protein (mg/l) | 2.61 | 4.46 | 1.69 (1.29; 2.10)\|\| |

Presented as means (standard deviation) or percentages

* Alive as of January 1, 2003
† Died during follow-up period
‡ Age and gender adjusted if applicable
§ CI = confidence interval
|| Significant ($p < 0.05$)
# High-density lipoprotein

with additional adjustment and remained significant. Each SD increase in venular diameters resulted in an 8% increased risk of mortality.

Stratification on age (table 5) showed that the association between venular diameters and mortality was more prominent in participants below 70 years (HR per SD increase: 1.18; 95% CI: 1.09-1.29). The corresponding non-significant HR for participants above 70 years was 1.03. Further adjustment for other cardiovascular risk factors attenuated the association in the younger age group, but did not alter the pattern of the association.

## DISCUSSION

We found that larger retinal venular diameters were associated with an increased risk of all-cause mortality independently of other cardiovascular risk factors. This association was more pronounced in the younger participants.

In interpreting these findings, we have to be aware of potential

**Table 2.** Hazard ratios* of all-cause mortality per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio

| | **All-cause mortality (n = 1762)** |
|---|---|
| Retinal arteriolar narrowing† | 1.00 (0.95-1.05) |
| Retinal venular dilatation‡ | 1.07 (1.02-1.12) |
| Retinal arteriolar-to-venular ratio§ | 1.07 (1.02-1.12) |

* Hazard ratios (95% confidence interval) were adjusted for age and gender
† Per standard deviation decrease in arteriolar diameters
‡ Per standard deviation increase in venular diameters
§ Per standard deviation decrease in arteriolar-to-venular ratio

confounding factors. Previously, we showed that larger venular diameters were related to cardiovascular risk factors such as more plaques in the carotid arteries, higher levels of total cholesterol, lower levels of HDL, higher levels of inflammatory markers (such as erythrocyte sedimentation rate and leukocyte count) and smoking.[15] Because these factors themselves are also independent predictors of mortality,[24,25] these factors may explain the observed association. In the present study, additional adjustment for all these risk factors in our multivariable models did not affect the relationship between venular dilatation and all-cause mortality. Our results thus suggest that venular dilatation might be an independent risk factor.

Both diabetic retinopathy and venous stasis retinopathy, the latter commonly seen in patients with occlusive disease of the carotid artery, are two examples of conditions in which retinal venular dilatation has been described.[26] It has been suggested that in these conditions persisting retinal hypoxia may lead to retinal venular dilatation. Successful treatment with retinal photocoagulation or carotid endarterectomy has been shown to be accompanied by a reduction in venular diameters, possibly due to less retinal hypoxia.[27] Vasodilatation of the venules and to a lesser extent arterioles has also been described in other vascular beds, including the renal and cerebral circulation.[28] Although the exact mechanisms underlying venular dilatation remain elusive, it could be that this dilatation is a general marker of poor health.

In a previous case-control study nested in the Beaver Dam Eye Study with 413 cases and 1198 controls, several retinal vessel signs such as arterio-venous nicking and focal arteriolar narrowing were related to an increased risk of cardiovascular mortality, as did a smaller AVR (OR: 1.5; 95% CI:

**Table 3**. Hazard ratios* of all cause mortality per quartile of baseline retinal vessel diameters or arteriolar-to-venular ratio

| Quartiles [range] | All-cause mortality (n = 1762) |
|---|---|
| Arteriolar diameter (μm) | |
| 4. [156.4-204.7] | 1.0 (Reference) |
| 3. [147.1-156.4] | 0.93 (0.81-1.07) |
| 2. [137.8-147.1] | 1.02 (0.89-1.16) |
| 1. [95.0-137.8] | 1.04 (0.91-1.18) |
| Venular diameter (μm) | |
| 1. [135.1-209.0] | 1.0 (Reference) |
| 2. [209.0-222.1] | 1.01 (0.89-1.15) |
| 3. [222.1-235.4] | 1.08 (0.95-1.24) |
| 4. [235.4-313.6] | 1.19 (1.05-1.37) |
| Arteriolar-to-venular ratio | |
| 4. [0.70-0.87] | 1.0 (Reference) |
| 3. [0.66-0.70] | 1.03 (0.90-1.18) |
| 2. [0.63-0.66] | 1.16 (1.02-1.33) |
| 1. [0.49-0.63] | 1.21 (1.06-1.37) |

* Hazard ratios (95% confidence interval) were adjusted for age and gender

1.1-2.1).[14] This effect was mainly present in the younger age category (43-74 years: OR: 1.9; 95% CI: 1.2-2.9) as was the case in our present study, whereas in persons above 75 years the AVR was not related to (OR: 1.0; 95% CI: 0.6-1.08).[14] When the same authors later analyzed this relationship with respect to the AVR in the entire Beaver Dam cohort (n = 4926), they could not replicate this finding, nor did they find an association with all-cause mortality.[13] Our data suggest that a smaller AVR was associated with an increased risk of all-cause mortality. Furthermore, retinal venular dilatation rather than arteriolar narrowing explained this association.

Previously, several studies reported an age-dependent association between retinal vessel signs and cardiovascular diseases.[11] An older study examining the association between retinal vessel signs and prevalent cardiovascular disease showed odds ratios of 3.7 to 6.4 in persons aged 35-54 years, but only 1.2 to 2.4 in participants aged 55-79 years.[29] Also, with respect to stroke, associations were present in a younger population (age range: 51-

**Table 4**. Multivariable adjusted hazard ratios* of all-cause mortality per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio

| | **All-cause mortality (n = 1762)** |
|---|---|
| Retinal arteriolar narrowing† | 0.97 (0.92-1.03) |
| Retinal venular dilatation‡ | 1.08 (1.02-1.14) |
| Retinal arteriolar-to-venular ratio§ | 1.04 (0.99-1.10) |

* Hazard ratios (95% confidence interval) were adjusted for age, gender, smoking, body mass index, total, HDL cholesterol, anti-hypertensive medication, carotid artery plaque score, diabetes mellitus, systolic and diastolic blood pressure, and C-reactive protein
† Per SD decrease in arteriolar diameters
‡ Per SD increase in venular diameters
§ Per SD decrease in arteriolar-to-venular ratio

72 years),[11] but were absent in older people (age range: 69-97 years).[30] Other studies have also reported that the predictive value of traditional cardiovascular risk factors, such as blood pressure and cholesterol were attenuated in older compared to younger people.[31-33] This pattern may reflect selective mortality. Persons who manifest retinal venular dilatation may be more likely to die before they reach age 70, leaving a select group of older persons whose biologic and genetic makeup protects them from processes associated with these retinal abnormalities.[14,31-33] Like all other studies, the cut-off value of 70 years was arbitrarily chosen. When we repeated the analyses with different cut-off values (such as 75 years), the results were the same, suggesting that the relationship between venular dilatation and mortality in the younger age category was robust.

A limitation of the present study is that we were not able so far to address the association of retinal vessel diameters with cause-specific mortality. Another limitation related to the retinal vessel measurement is that photographs were not taken synchronized on the cardiac cycle, so there might be variation in vessel diameter due to pulsatility.[15] Because photography was independent of any participant characteristics, this will have caused random misclassification. Strengths of the current study are its population-based prospective design, large study sample, and standardized procedures for retinal vessel measurements. Furthermore, we adjusted the diameters for the refraction using Littmann's formula, enabling us to use the separate arteriolar and venular diameter sum values.

In conclusion, our results suggest that retinal venular dilatation is a new independent predictor of all-cause mortality in the general population.

**Table 5**. Hazard ratios* of all-cause mortality per standard deviation difference in baseline retinal vessel diameters or arteriolar-to-venular ratio stratified on age

| | Model I\|\| | Model II# |
|---|---|---|
| **< 70 years (n = 587 cases)** | | |
| Retinal arteriolar narrowing† | 1.01 (0.93-1.10) | 0.99 (0.90-1.09) |
| Retinal venular dilatation‡ | 1.18 (1.09-1.29) | 1.11 (1.01-1.23) |
| Retinal arteriolar-to-venular ratio§ | 1.20 (1.10-1.31) | 1.09 (0.99-1.21) |
| **≥ 70 years (n = 1175 cases)** | | |
| Retinal arteriolar narrowing† | 1.00 (0.94-1.05) | 0.96 (0.90-1.03) |
| Retinal venular dilatation‡ | 1.03 (0.97-1.09) | 1.06 (0.99-1.13) |
| Retinal arteriolar-to-venular ratio§ | 1.02 (0.97-1.08) | 1.02 (0.95-1.08) |

* Hazard ratios (95% confidence interval)
† Per SD decrease in arteriolar diameters
‡ Per SD increase in venular diameters
§ Per SD decrease in arteriolar-to-venular ratio
|| Model I: adjusted for age and gender
# Model II: additionally adjusted for smoking, body mass index, total, HDL cholesterol, anti-hypertensive medication, carotid artery plaque score, diabetes mellitus, systolic and diastolic blood pressure, and C-reactive protein

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# *Part VI*

# *General Discussion and Summary*

# Chapter 6.1

# General Discussion

The main objective of the work described in this thesis was to enhance our knowledge on associations between baseline retinal arteriolar and venular diameters both separately and combined in the AVR, and ophthalmological, neurological and systemic diseases in the elderly. For this purpose, I described a number of studies that were performed within the context of the Rotterdam Study.[1] In this discussion, some general methodological issues that came up during these studies will be described. Furthermore, the main findings are summarized and placed in a broader perspective. Finally, views on further research are presented.

## METHODOLOGICAL CONSIDERATIONS

### Study design

The Rotterdam Study is a prospective population-based cohort study. Its aim is to investigate the incidence and determinants of chronic disabling diseases in the older population. All inhabitants (n = 10,275) of a district of the city of Rotterdam aged 55 years or over were invited in random order to the study, and 7983 actually participated (overall response rate 78%).[1] Because the ophthalmic part became operational after the pilot study had already

started, 6780 participants underwent ophthalmic examination.[2] Baseline home interview and examinations were performed from 1990 to 1993, followed by follow-up screenings in 1993-1994, 1997-1999 and 2003-2004. In addition, the total cohort was and is continuously monitored for morbidity, mortality and medication use through automated linkage of the study database with files from general practitioners, the municipality and the pharmacies.

In the previous chapters each individual study has been discussed as to its merits and limitations. Here I would like to discuss two main issues from a more general perspective. The first deals with precision of retinal vessel measurements, and the second with internal validity. Precision involves minimizing random error. Random error may lead to both imprecise effect estimates (broad confidence intervals) or underestimated point estimates (regression dilution bias). Internal validity pertains to limiting systematic error or bias of which three types can be identified: selection bias, information bias and confounding.[3]

**Precision of retinal vessel measurements**

For retinal vessel measurements we used simultaneous stereoscopic fundus color photographs centered on the optic disc, which were taken at the baseline eye examination.[4] For each participant the digitized image of one eye with the best quality was analyzed with the Retinal Vessel Measurement System.[5]

We tried to reduce random error by having a large overall sample size (n = 5674). Moreover, we performed our measurements on transparencies obtained after pharmacological mydriasis with a $20^0$ field leading to higher magnification. In contrast, two previous studies used $45^0$ photographs without pharmacological mydriasis, whereas another used $30^0$ photographs through dilated pupils.[5-7] Third, consensus sessions and between-grader comparisons were performed regularly to safeguard high reproducibility. Pearson's correlation coefficients for inter-grader agreement were for arteriolar diameters 0.67-0.80, for venular diameters 0.91-0.94 and for AVR 0.75-0.84. For intra-grader agreement the corresponding figures were 0.69-0.88, 0.90-0.95 and 0.72-0.90. Fourth, we used the improved formulas,[8] that used the six largest arterioles or venules to calculate the sum values, instead of the original ones, that used all measured vessel diameters.[5] Because larger vessels were more easily measurable this further increased the precision. Fifth, the variability caused by the refractive status of the eye was eliminated by correcting the sum vessel diameters with Littmann's formula in case of ametropia.[9]

Despite these efforts to minimize random error, precision could have been further improved. Photographs were not taken synchronized on the cardiac cycle, so there might be variation in vessel diameter due to pulsatility. A variation of 2-17% in vessel diameter has been described.[10] However, because photography was independent of any person characteristics, this will have caused non-differential misclassification, leading to an underestimation of the risks.

## Internal validity

### *Selection bias*

An important threat to validity comes from selection bias. Its main characteristic is that the relation between determinant and disease is different for those who participate and those who do not. A population-based design greatly reduces the potential for selection bias, because inclusion of persons is random and therefore not distorted by determinants related to disease. The response at baseline, however, was not complete, because 12% of those who underwent fundus photography did not have gradable transparencies. This group probably contained more persons with physical or mental disabilities or lens opacities that contributed to the poor quality of the transparencies. However, there were only small differences in cardiovascular risk factors between those with and without gradable transparencies, suggesting a limited role for selection bias.

Participation rates at follow-up were not uniform for all outcomes. Because the entire cohort was continuously monitored for major morbidity (such as stroke and dementia) and mortality, the follow-up was nearly complete for these outcomes, minimizing the possibility of selection bias. However, for others (e.g. age-related eye diseases), a person's participation in the follow-up examinations was crucial, because the files of the general practitioners were not detailed enough to make a reliable diagnosis. Although non-participants at follow-up showed statistically significant differences compared to the participants with respect to their cardiovascular profile at baseline, the baseline retinal vessel diameters were not different, suggesting a limited role for selective non-response.

### *Information bias*

Another threat to validity is information bias, which results from misclassification of the outcome or the determinant. Misclassification of the outcome that is related to the determinant, or vice versa, results in differential misclassification, and possibly a distortion of the findings. This distortion

cannot be adjusted for in the analyses. The prospective design of our studies, with the measurement of determinant before the onset of disease, excludes the possibility of differential misclassification of the determinant. The same holds for missing data caused by failure of equipment or absence of a research assistant. Because these data were collected at baseline, their absence cannot be associated with the incident outcomes. Differential misclassification of outcomes is also unlikely, because persons who performed these examinations were masked for exposure status of the cases. In our studies, the measurements of the determinant, outcome and risk factors were independently performed in a masked way from each other. Therefore, it is very unlikely that misclassification, if any, would have been differential and hence would have resulted only in random error as has already been mentioned above.

### *Confounding*

Confounding may be considered a confusion of effects. A potential confounder is associated with both the determinant and the outcome, and is not an intermediate in the causal pathway leading to the outcome variable under study. One can neutralize the effect of a confounder by measuring it carefully and adjusting for it in the statistical analyses. Even though I adjusted for many potential confounders, the possibility of insufficient measurement of confounders (residual confounding) or the presence of unknown confounders should always be considered in observational studies.

## MAIN FINDINGS

### Are retinal arteriolar or venular diameters related to markers for cardiovascular diseases?

A lower arteriolar-to-venular diameter ratio (AVR) was suggested to reflect generalized arteriolar narrowing and was associated with an increased risk of cardiovascular and cerebrovascular diseases.[11-13] It remained unclear, however, what exactly the separate arteriolar and venular diameters contributed to the AVR and what kind of vascular pathology this ratio precisely reflected.

A striking finding in our study was that retinal venular diameters are not stable, as was usually thought. Larger venular diameters were related to more atherosclerosis (as measured by the ankle-arm index, aortic calcifications and carotid plaque-score), higher leukocyte count, higher erythrocyte sedimentation rate, higher total cholesterol, lower HDL levels, higher waist-

to-hip ratio and smoking.[4] So far, one study reported that a smaller AVR was also related to plaques in the carotid artery, smoking and higher levels of inflammatory markers.[14] Based on our study, we think that venular dilatation might underlie these reported associations. It has been hypothesized that increase in venular diameters may result from retinal hypoxia.[15] Venular and to a lesser extent arteriolar dilatation has been described not only in the early stages of diabetic retinopathy, but also in venous stasis retinopathy, which is commonly seen in patients with occlusive disease of the carotid artery.[16,17] Treatment with carotid endarterectomy or retinal photocoagulation leads to reduction of venular diameters, probably due to less retinal hypoxia.[18,19] However, the exact mechanisms leading to venular dilatation remain elusive.

Our data confirm that elevated blood pressures were associated with smaller arteriolar diameters. The decrease in arteriolar diameters not only reflects vasoconstriction but also intimal thickening, hyalinization and sclerosis.[5,7,20,21] We found a smaller decrease in arteriolar diameter (1.1μm and 2.1μm per 10-mmHg increase in systolic and diastolic blood pressure) than previously reported (1.9μm and 4.3μm).[7] This smaller decrease could be explained by the older age distribution of our cohort (55-99 years) compared to the Atherosclerosis Risk in Communities (ARIC: 48-76 years) and the Blue Mountains cohorts (49+ years). Also, in our study the association of the arteriolar diameters was strongest in the younger persons.[4] In the older age groups the vessels probably become progressively rigid and lose their ability to adequately react to blood pressure changes.[22,23]

In summary, the idea that a lower AVR overall reflects only generalized arteriolar narrowing is no longer tenable. These data indicate that the venular diameters do not remain constant in different pathological conditions and may play their own independent role in predicting cardiovascular disease. We proposed that the arteriolar and venular diameters should be analyzed separately with regard to ophthalmological, neurological and systemic diseases in the elderly (chapter 2).[4]

**Retinal vessel diameters and age-related eye diseases**

In part III of this thesis, we examined the role of vascular pathology, including retinal vessel diameters, in the etiology of aging macular disease (AMD). According to a vascular hypothesis, AMD is a consequence of stiffening of the sclera and choroidal vessels, due to aging, atherosclerosis and other causes, impairing the choroidal blood flow.[24-26] This process would interfere with the high metabolic rate of the retinal pigmented epithelium and photoreceptors, and lead to the development of subretinal deposits (drusen),

pigment abnormalities, and finally to the blinding end-stages of atrophic or neovascular AMD.[27]

Most epidemiological studies have addressed the vascular hypothesis by studying classical cardiovascular risk factors like blood pressure, as well as clinical manifestations of atherosclerosis such as myocardial infarction.[28-30] However, few of these studies were population-based and prospective in design. In the Rotterdam Study, we found that the risk of AMD increased by 8% for each 10-mmHg increase in systolic blood pressure. The presence of sub-clinical atherosclerosis, especially in the carotid artery, also increased the risk of AMD in a dose-dependent manner. Our data further support the vascular hypothesis in the pathogenesis of AMD (chapter 3.1).[31] A drawback of this approach might be that these markers of vascular pathology are related to larger blood vessels outside the eye and may not represent local ocular abnormalities.[27]

To further explore this hypothesis, the role of the retinal circulation was examined in relation to AMD (chapter 3.2).[32] Our data showed that neither smaller arteriolar nor larger venular diameters were associated with the risk of AMD.[32] The Blue Mountains Eye Study and the Beaver Dam Eye Study also did not find an association between retinal vessel diameters and incident AMD (odds ratio [OR] 1st versus 5th quintile of arteriolar diameters: 1.4 (95% confidence interval [CI]: 0.3-5.7) and 1.8 (95% CI: 0.6-5.4)).[33,34] How can these results be matched with those of chapter 3.1, which support the vascular hypothesis?

A short description of some local differences between the choroidal and retinal circulations could aid in resolving this inconsistency. The choroidal circulation is responsible for the nourishment of the retinal pigmented epithelium and the outer layers of the sensory retina, and is characterized by a high blood flow and a low arterio-venous difference in oxygen content.[35] The retinal circulation in contrast nourishes only the inner retina and is characterized by a low blood flow and high oxygen consumption.[35] Furthermore, apart from the autonomous innervation there seems to be, unlike the retinal vasculature, no auto-regulatory mechanisms to maintain choroidal blood flow.[35,36] The choroidal arteries are short, thereby transmitting the systemic blood pressure more directly to the choriocapillaris. Because of these differences the choroidal vessels might be more vulnerable to hypertensive damage than their retinal counterparts, as is also clinically known from disorders as pre-eclampsia or Elshnig's spots in a hypertensive crisis.[36-38] These differences might explain the lack of an association between retinal vessels and the risk of AMD.

It has also been suggested that hyperopia can lead to AMD by increasing choroidal outflow resistance resulting in impaired retinal pigment epithelial function.[26,39-41] Sofar, several studies showed conflicting results on this topic.[29,30,41-43] We found that each standard deviation increase in refractive error towards hyperopia was associated with a 5-9% increased risk of AMD (chapter 3.3).[44] In general, hyperopic eyes have thicker and more rigid sclerae.[45] This generalized stiffness of the sclera may cause an increase in resistance of the choroidal venous outflow,[24,26] and thereby prevent exchange of nutrients and metabolic products across the retinal pigmented epithelium resulting in drusen formation and thickening of Bruch's membrane.[46,47] In summary, our data provide evidence in favor of the vascular hypothesis in the etiology of AMD probably through an impaired choroidal circulation. Moreover, this association is most likely a causal one, considering the longitudinal design of these studies.

Another age-related eye disease in which vascular factors have been hypothesized to play a role in its development is open-angle glaucoma (OAG). It is characterized by progressive loss of retinal ganglion cells and their axons, resulting in glaucomatous optic neuropathy with corresponding visual field loss. In this case, perfusion of the retinal ganglion cell layer or the optic nerve head seems to be impaired.[48-50] Retinal arterioles vascularize the inner part of the retina (including the retinal ganglion cell layer) and the surface layer of the optic nerve head, whereas the short posterior ciliary arteries are responsible for blood supply to the optic nerve head.[51]

A case-control study showed that OAG patients had significantly smaller arteriolar diameters, measured on optic disc photographs, than age-matched control persons.[52] Another one with a population-based cross-sectional design, confirmed these findings.[53] Due to the cross-sectional design of these studies an important etiological question remained unanswered: whether an impaired retinal circulation plays a causative role in the pathophysiology of OAG or occurs as a consequence of decreased metabolic demand after retinal ganglion cell loss.[50] Clinically, it is known that reduced retinal blood perfusion, such as in central retinal artery occlusion or non-arteritic anterior ischemic optic neuropathy, usually does not lead to glaucomatous cupping.[54,55] However, there are no prospective data on the relationship between retinal vascular factors and incident OAG. We showed that neither retinal arteriolar nor venular diameters were related to an increased risk of incident OAG. In line with these findings, retinal vessel diameters did not predict incident glaucomatous optic disc changes (chapter 3.4).[56]

In contrast to the retinal vessels, several studies have attributed a

more prominent role to the short posterior ciliary arteries, which are the main sources of blood supply to the optic nerve head.[57-59] It has been reported that the auto-regulation in the short posterior ciliary arteries seems to be less efficient than in the retinal circulation.[50,51] Also, in contrast to the retinal vessels, the optic nerve head vasculature has no proper blood-tissue barrier, making it more sensitive to fluctuating levels of vaso-active molecules (such as angiotensin-II).[50] Because of these local differences the short posterior ciliary arteries may be more vulnerable to vascular damage than the retinal vessels. The data available up to date, including our prospective data, provide evidence against a retinal vascular cause in the pathogenesis of retinal ganglion cell loss, and the subsequent development of glaucomatous visual field defects.

**Retinal vessel diameters, cerebrovascular disease and dementia**

Apart from the role of retinal vessels in ocular diseases, these vessels have been suggested to provide information on vascular pathology of the brain, because they share many features with the cerebral vessels such as blood-tissue barrier, auto-regulation and embryological origin.[60,61] Most studies that examined retinal vessel signs in relationship to cerebral pathology were hospital-based and conducted in patients with rare hereditary diseases involving small vessels like cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) or hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS).[62-64] More recently, the ARIC investigators showed that a decreasing AVR was associated with an increased risk of cerebrovascular diseases.[11,65] Because the authors did not present arteriolar and venular diameters separately, we do not know if they examined these associations.

In the Rotterdam Study, larger venular diameters were related to an increased risk of stroke and its major subtype cerebral infarction (chapter 4.1). In a random sample of non-demented elderly with MRI scans, we also examined the association between retinal venular diameters and markers of cerebral small vessel disease, including white matter lesions and lacunar infarcts. Larger venular diameters were also related to the progression of periventricular and subcortical white matter lesions, particularly to marked lesion progression. Furthermore, larger venular diameters were associated with incident lacunar infarcts (chapter 4.2). Cerebrovascular abnormalities play an important role not only in vascular dementia, but also in the clinical course and progression of Alzheimer's disease.[66,67] In chapter 4.3, we examined the association between retinal vessel diameters and the risk of dementia. Again,

we found larger venular diameters to be associated with an increased risk of dementia, including its major subtypes Alzheimer's disease and vascular dementia. All associations were independent of other known cardiovascular risk factors.

Retinal venular dilatation is thought to occur in response to retinal hypoxia as described in diabetic retinopathy and the less well-known venous stasis retinopathy.[16,17] Based on these observations, we hypothesized that venular dilatation might also be a general cerebral feature reflecting a decreased blood flow and diffuse cerebral ischemia, making the brain tissue more prone to the development of cerebrovascular diseases. A disturbance in the cerebral venous outflow could lead to elevated venous pressure and eventually to insufficiency of cerebral blood flow and hypoperfusion.[68-70] In one histo-pathological study, brains with severe white matter lesions showed wall thickening and obstruction of the periventricular veins, a condition known as periventricular venous collagenosis.[71,72] The authors suggested that these alterations resulted in an increased venous pressure, venular dilatation, and venular blood-brain barrier disruption. This venous insufficiency could result in ischemic stress from impaired clearance of cellular metabolites in the deep white matter eventually leading to white matter lesions and more extensive vascular damage.[72,73]

Also, a disturbed cerebral venous outflow may interfere with the drainage of cerebrospinal fluid and interstitial fluid, both of which are important in maintaining a constant external environment for both neurons and glia.[74] Increased resistance to cerebrospinal fluid could lead to decreased clearance of toxic molecules such as amyloid-beta peptide.[75,76] The resulting amyloid-beta depositions (e.g. in arterial walls) and accumulation of other metabolic products would enhance chronic cerebral hypoperfusion eventually contributing to tissue damage.[75,76] Despite the fact that the cerebral venous system may be one of the most important factors that guarantees normal cerebral function,[77] thus far little is known about the patho-physiology of venous abnormalities and their role in cerebrovascular diseases and dementia. Furthermore, it is unkown to what extent such cerebral venular abnormalities might be reflected in the retinal venules.

Smaller arteriolar diameters are related to higher blood pressures,[4] possibly due to vasoconstriction, intimal thickening and medial hyperplasia.[5,7,20,21] Therefore, we hypothesized that smaller arteriolar diameters would be related to the risk of cerebrovascular diseases. However, our study did not show any association between smaller arteriolar diameters and risk of cardiovascular diseases and dementia. How should we interpret these negative findings? The

lack of an association could be due to the fact that in conditions reflecting retinal or cerebral hypoxia, the arterioles still possess, despite increased arterial stiffness, the ability to dilate, although less pronounced than in the venules. These opposing effects in the same arterioles, narrowing and widening, might result in no apparent association.

Taken together, we found retinal venular dilatation to be associated with an increased risk of cerebrovascular diseases and dementia. These associations were independent of other known cardiovascular risk factors.

**Is aging macular disease an ocular manifestation of Alzheimer's disease?**

Apart from retinal vessel diameters, we examined if other retinal signs, such as those seen in AMD, were associated with Alzheimer's disease. Because both these diseases are characterized by extra-cellular deposits and irreversible neuronal cell loss, several studies linked AMD to Alzheimer's disease.[78-82] In the Rotterdam Study, preliminary data suggested that AMD predicted the two-years risk of Alzheimer's disease in participants above 75 years.[78] Because we now have a longer follow-up, we re-examined this association in chapter 4.4. We found no evidence for an increased risk of dementia or its major subtype Alzheimer's disease among persons with AMD at baseline. Furthermore, we examined cross-sectionally a possible association between AMD and structural brain changes on MRI including hippocampal, amgydalar and generalized brain atrophy that may be considered to be preclinical markers of Alzheimer's disease. Also, no relation was found between AMD and different measures of brain atrophy. These data did not support our previous findings that AMD may be associated with Alzheimer's disease.

**Retinal vessel diameters, systemic diseases and mortality**

Studies on the ARIC and Beaver Dam cohorts showed that a smaller AVR at baseline was related to incident hypertension.[83,84] The Blue Mountains Eye Study (n = 1319) reported the relationship between separate arteriolar diameters and incident hypertension (OR 1$^{st}$ versus 5$^{th}$ quintile: 2.4; 95% CI: 1.6-3.5).[85] These prospective data provide evidence that generalized arteriolar narrowing may already precede the development of hypertension rather than occur as a secondary adaptation.[86,87] In our study, we confirmed this association between smaller arteriolar diameters and incident hypertension (chapter 5.1). Because smaller venular diameters (OR per SD decrease: 1.16; 95% CI: 1.05-1.28) also increased the risk of hypertension, the magnitude of the association with the AVR (OR: 1.29; 95% CI: 1.17-1.43) was smaller

than with the arteriolar diameters only (OR: 1.42; 95% CI: 1.29-1.57). These data thus suggest that in order to determine the risk of hypertension, the arteriolar diameters should be assessed independently. In contrast, in clinical ophthalmoscopy arteriolar diameters are usually gauged against venular ones in order to assess generalized arteriolar narrowing. Our data imply that this approach may lead to spurious results with respect to assessing the extent of arteriolar narrowing and thereby the risk of hypertension.

The ARIC investigators also reported an association between AVR and diabetes mellitus. Each standard deviation decrease in the AVR was related to a 26% increased risk of diabetes mellitus.[12] Based on these data the authors concluded that generalized arteriolar narrowing may play a role in the initial development of diabetes.[12] Studying the arteriolar and venular diameters separately is especially important in this case, because retinal venular dilatation has been reported to be present already in the pre-diabetic stage of hyperglycemia.[88-90] Increase in venular diameters is thought to result from retinal hypoxia and lactate accumulation.[15,91] In a case-control study, 266 patients with juvenile diabetes mellitus had significantly larger mean venular diameters (396.5μm) than 129 controls (368.0μm).[89] Also, a population-based cohort study on 996 diabetic patients showed an association between larger venular diameters and 4-years progression of retinopathy (relative risk [RR] 4$^{th}$ versus 1$^{st}$ quartile: 2.3; 95% CI: 1.4-4.0) and 14-year incidence of proliferative retinopathy (RR: 4.3; 95% CI: 1.5-12.2).[15] We investigated if smaller arteriolar or larger venular diameters were associated with incident impaired fasting glucose or diabetes mellitus. The risk of impaired fasting glucose and diabetes mellitus with AVR was largely explained by venular dilatation rather than arteriolar narrowing (chapter 5.2).

Finally, also with respect to the association between the AVR and mortality, data were inconsistent.[92,93] In a previous case-control study nested in the Beaver Dam Eye Study with 413 cases and 1198 controls, a lower AVR was related to an increased risk of cardiovascular mortality (OR: 1.5; 95% CI: 1.1-2.1).[93] This effect was mainly present in the younger age category (43-74 years: OR: 1.9; 95% CI: 1.2-2.9), whereas in persons above 75 years the AVR was not related to cardiovascular mortality (OR: 1.0; 95% CI: 0.6-1.08).[93] When the same authors later analyzed this relationship with respect to the AVR in the entire Beaver Dam cohort (n = 4926), they could not replicate this finding, nor did they find an association with all-cause mortality.[92] Our data again revealed that larger venular diameters were related to an increased risk of all-cause mortality (chapter 5.3). Previously, several studies reported an age-dependent association between retinal vessel signs and cardiovascular

diseases.[11] One study examining the association between retinal vessel signs and prevalent cardiovascular disease showed odds ratios of 3.7 to 6.4 in persons aged 35-54 years, but only 1.2 to 2.4 in participants aged 55-79 years.[94] Others also reported that the predictive value of traditional cardiovascular risk factors, such as blood pressure and cholesterol were attenuated in older compared to younger people.[95-97] Stratification on age showed that the association between venular diameters and mortality was more prominent in participants below 70 years. This pattern may reflect selective mortality. Persons who manifest retinal venular dilatation may be more likely to die before they reach age 70, leaving a select group of older persons whose biologic and genetic makeup protects them from processes associated with these retinal abnormalities.[93,95-97] In conclusion, our results suggest that retinal venular dilatation is a new independent risk factor for all-cause mortality in the general population.

## FUTURE PERSPECTIVES

The most important and consistent finding emerging from this thesis is that retinal venular diameters do not remain constant and that venular dilatation is independently related to an increased risk of cerebrovascular and systemic diseases in the elderly. Still, there are many unresolved questions. Because these results are based on a single measurement of retinal venular diameter on baseline fundus photographs of each individual, it is not known what kind of changes occur in venular diameters over time within the same person. In order to quantify change in venular diameters, I propose to measure vessel diameters, using the same semi-automated system, on fundus photographs taken at the follow-up examinations. This approach will allow us to quantify changes in venular diameters within the same individual and to examine the determinants of these changes. Alternatively, this can be done in a more time-efficient way by using a fully automated system for retinal vessel measurement, which will hopefully become available in the near future.

Initially markers of atherosclerosis (ankle-arm index, carotid artery plaque score) and inflammation (leukocyte count, erythrocyte sedimentation rate), which were cross-sectionally related to larger venular diameters, can be related to changes in venular diameters. Other possible determinants that could be considered are: new inflammatory markers (such as C-reactive protein, lipoprotein-associated phospholipase A2), markers of vessel stiffness, arterial oxygen saturation, genetic polymorphisms (angiotensin converting enzyme, apolipoprotein E, nitric oxide synthesis) and medication use (beta-blockers, diuretics, ACE-inhibitors, calcium-antagonists, nitrates). Experimental studies

utilizing animal models may be needed to elucidate the exact mechanisms through which these factors lead to venular dilatation.

Unraveling these mechanisms may also provide explanations for the associations we described with cerebrovascular and systemic diseases. The quantified changes in retinal arteriolar and venular diameters could also be used to examine whether these changes are related to an increased risk of cerebrovascular and systemic diseases. With respect to cerebrovascular diseases, another approach would be to examine directly the cerebrovenous system. For a long time, digital subtraction angiography was considered the method of choice in evaluating the intracranial venous system in a clinical setting. For larger, preferentially population-based studies, this technique is difficult to implement, because of the necessity for contrast-medium injection. The advent of magnetic resonance (MR) imaging and the introduction of MR angiography techniques provided new possibilities for non-invasive visualization of the cerebral veins and sinuses.[98] Two such non-invasive techniques are the time-of-flight (TOF) MR angiography and phase-contrast MR angiography. These state-of-the-art imaging methods may facilitate examination of the cerebrovenous system in population-based studies, leading to a better understanding of its anatomical complexity and its role in cerebrovascular diseases.

Because there are obvious differences between on the one hand retinal and on the other choroidal and optic nerve head circulations,[35,36,50] one has to measure local vascular changes to investigate the role of vascular pathology in age-related eye diseases such as AMD and OAG. Confocal scanning laser Doppler flowmetry and color Doppler ultrasound imaging are two examples of non-invasive techniques that are capable of measuring blood flow in the retrobulbar vessels, such as choroidal and posterior ciliary vessels.[50,51,99] My suggestion would be to use these non-invasive techniques to measure specific local ocular blood flow in an on-going population-based study and examine the incidence of AMD or OAG. Furthermore, these techniques could also be used to measure the blood flow in the ophthalmic or the retinal vessels. Like the retinal vessel diameters, these measurements then could also be used as a proxy for cerebral hemodynamics.

In conclusion, the retinal venular system should no longer be considered an invariable collection of efferent vessels. This should stimulate future research into the mechanisms leading to venular abnormalities as these may provide exciting new directions into the pathogenesis of diseases in the elderly.

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# Chapter 6.2

## Summary

Since the late 19th century, caliber changes in the retinal vessels of the eye were recognized as markers of vascular changes throughout the body and were used to predict life prognosis. Different classifications mainly depended on qualitative assessment of retinal vessels using ophthalmoscopy. Ophthalmoscopy has a poor reproducibility and it was common to gauge the diameters of the small arteries (arterioles) against those of the small veins (venules). Recently, a semi-automated system was developed to measure the vessel diameters on photographs of the retina. A lower ratio of the arteriolar to venular diameters (AVR) was attributed to generalized arteriolar narrowing and it was suggested that this ratio might predict incident cardiovascular diseases independently of known cardiovascular risk factors. It remained unclear, however, how exactly the separate arteriolar and venular diameters contributed to the AVR and what kind of vascular pathology this ratio precisely reflects. Furthermore, it was unknown if the associations with cardiovascular diseases were due to arteriolar narrowing, venular dilatation, or a combination of both.

Thus, the main objective of the work presented in this thesis was to study the associations between separate arteriolar and venular diameters and the risk of diseases in the elderly, possibly related to changed blood supply.

All studies in this thesis were based on data from The Rotterdam Study, which was initiated in 1990-1993. This is a prospective, population-based cohort study among 7983 persons aged 55 years or over, who lived in a well-defined district of the city of Rotterdam, The Netherlands.

A historical overview of vascular classifications and a general introduction is presented in **chapter 1**. **Chapter 2** describes a study in which we examined associations between retinal arteriolar or venular diameters and their AVR and markers of sub-clinical cardiovascular disease, disease that till then had not resulted in symptoms apparent to the participants. Larger venular diameters were related to more atherosclerosis, higher white blood cell counts, higher sedimentation rates of red blood cells, higher total cholesterol and lower HDL levels, higher ratio between waist and hip circumference and smoking. We confirmed that smaller arteriolar diameters were related to higher blood pressures. Because we found that venular diameters showed more variation than previously thought, we proposed to analyze in the future arteriolar and venular diameters separately with respect to diseases in the elderly.

**Part III** focuses on ophthalmological diseases. **Chapter 3.1** describes the role of vascular factors in the development of aging macular disease (AMD), a degenerative disease affecting the center of the retina, the macula lutea and the area with highest visual acuity. AMD is the leading cause of incurable blindness and visual impairment in the Western world. We found that markers of sub-clinical atherosclerosis were associated with an increased risk of AMD, as were elevated blood pressures. These data provide evidence for the hypothesis that AMD is partially caused by vascular disease. To further explore this vascular hypothesis, in **chapter 3.2** the role of the retinal circulation was examined in relation to AMD. Our data showed that neither smaller retinal arterioles nor larger venules were associated with the risk of AMD. This agrees with the present paradigm, that AMD might be caused by disturbed blood flow in structures lying underneath the retina: namely the choroidal circulation and the retinal pigment epithelium. Moreover we examined in **chapter 3.3** if hyperopia (long-sightedness) that may lead to impaired choroidal circulation presumably due to increased rigidity or thickness of the outer coat of the eyeball (sclera), is associated with AMD. We found that hyperopic eyes are at an increased risk of developing AMD. **Chapter 3.4** addresses the issue whether retinal vessels may play a role in the development of another eye-disease, namely primary open-angle glaucoma. We showed that neither arteriolar nor venular diameters were related to an increased risk of glaucoma. In line with these findings, retinal vessel diameters

did not predict changes in the optic nerve in the eye, nor did they predict changes in the width of the neural rim.

**Part IV** focuses on neurological diseases. In **chapter 4.1**, we showed that larger venular diameters were related to an increased risk of stroke and its major subtype cerebral infarction. In **chapter 4.2**, data from a random sample of non-demented elderly with MRI scans were presented. We also found that larger venular diameters were related to the progression of white matter lesions, both around the brain ventricles (periventricular) and under the cerebral cortex (subcortical). Furthermore, persons with larger venules tended to have more new lacunar infarcts. Because vascular abnormalities in the brain also play an important role not only in vascular dementia, but also in Alzheimer's disease, we examined the association between retinal vessel diameters and dementia in **chapter 4.3**. Again, we found that larger venular diameters were associated with an increased risk of dementia, including Alzheimer's disease and vascular dementia. All associations were independent of other known cardiovascular risk factors. Apart from retinal vessel diameters, we examined in **chapter 4.4** if other retinal signs, such as those seen in AMD, were associated with Alzheimer's disease. Our data did not support the notion that AMD is an ocular manifestation of Alzheimer's disease or vice versa.

**Part V** explores the associations between retinal vessel diameters and the risk of systemic diseases. In **chapter 5.1**, we confirmed that smaller arteriolar diameters were related to an increased risk of high blood pressure (hypertension). These data provide evidence that generalized arteriolar narrowing may already precede the development of systemic hypertension. In **chapter 5.2**, we found that dilatation of the venules rather than narrowing of the arterioles largely explained the risk of impaired fasting glucose and diabetes mellitus with smaller AVR. In the last chapter of this part (**chapter 5.3**), we also found that larger venular diameters were related to an increased risk of all-cause mortality.

Finally, in **chapter 6**, the methodology of the studies, the main findings and suggestions for further epidemiological research are discussed. My final conclusions were that venules do not remain constant in different pathological conditions and may play an independent role in determining the risk of cerebrovascular and cardiovascular diseases. The identification of mechanisms underlying venular abnormalities may provide new insights into the patho-physiology of diseases in the elderly.

# Chapter 6.3

## Samenvatting

Sinds het einde van de 19de eeuw, wordt aangenomen dat kaliber veranderingen in de vaten van het netvlies (retina) in het oog kenmerkend zijn voor identieke vaatveranderingen elders in het lichaam. Lang werden deze vaatveranderingen gebruikt om de levensverwachting te voorspellen. Verschillende classificaties werden opgesteld, voornamelijk gebaseerd op kwalitatieve beoordeling van de retinavaten met behulp van oogspiegelen. Oogspiegelen heeft een matige reproduceerbaarheid en het was gebruikelijk hierbij de diameters van de kleine slagaders (arteriolen) af te meten tegen die van de kleine aders (venulen).

Recent werd een deels geautomatiseerd systeem ontwikkeld voor het meten van de vaatdiameters op netvliesfoto's. Een kleinere verhouding (ratio) tussen de arteriolaire en venulaire diameters (AVR) werd toegeschreven aan een vernauwing van de kleine slagaders en er werd gesuggereerd dat een kleinere AVR gepaard ging met een hoger risico op het optreden van hart- en vaatziekten, onafhankelijk van andere bekende risicofactoren voor hart- en vaatziekten. Het was onduidelijk wat de precieze bijdrage van de losse arteriolaire en venulaire diameters aan de uitkomst van de AVR was en wat voor ziekteprocessen (pathologie) deze AVR precies weerspiegelde. Bovendien was het onbekend of het verband met hart- en vaatziekten het gevolg was van

arteriolaire vernauwing, venulaire verwijding of een combinatie van beide.

De hoofddoelstelling van dit proefschrift was het bestuderen van het verband tussen de diameters van de arteriolen en venulen in het netvlies en de kans op ziekten op oudere leeftijd, waarvan werd aangenomen dat deze mogelijk gerelateerd waren aan een gestoorde bloedvoorziening. Alle studies zijn gebaseerd op data afkomstig uit het "Erasmus Rotterdam Gezondheid en Ouderen" (ERGO) onderzoek, in het Engels "The Rotterdam Study" genaamd. Dit is een prospectief bevolkingsonderzoek, waaraan tussen 1990 en 1993 7983 personen van 55 jaar en ouder, wonend in de Rotterdamse wijk Ommoord, deelnamen.

Na een historisch overzicht over vaatclassificaties en een algemene inleiding in **hoofdstuk 1**, wordt **hoofdstuk 2** gewijd aan het verband tussen enerzijds de diameters van de slagaders en aders in het netvlies en hun onderlinge verhouding (AVR) en anderzijds markers van subklinische hart- en vaatziekten, dat zijn hart- en vaatziekten die nog niet tot merkbare klachten aanleiding hadden gegeven. Grotere diameters van de aders waren gerelateerd aan meer slagaderverkalking, hogere aantallen witte bloedlichaampjes, hogere bezinkingssnelheid van de rode bloedlichaampjes, hoger totaal cholesterol en lager HDL cholesterol gehalte, grotere taille omvang ten opzichte van heupomvang, en aan roken. We bevestigden dat kleinere slagaderlijke diameters gerelateerd waren aan hogere bloeddrukken. Omdat bleek dat de diameters van de aders meer variatie vertoonden dan tevoren aangenomen werd, stelden wij voor om in verband met deze ouderdomsziekten de diameters van de slagaders en aders afzonderlijk te analyseren.

**Deel III** richt zich op oogziekten. In **hoofdstuk 3.1** wordt de rol van vasculaire factoren bij het ontstaan van afsterven van de gele vlek (macula lutea) in het centrum van het netvlies, de zogenaamde ouderdomsmaculadegeneratie (OMD), de belangrijkste oorzaak van permanente slechtziendheid in de westerse wereld, beschreven. We vonden dat markers van subklinische slagaderverkalking gerelateerd waren aan het risico van OMD. Bovendien, gingen hoge bloeddrukken gepaard met een hoger risico van OMD. Deze resultaten ondersteunen de hypothese dat OMD deels door vaatziekten veroorzaakt kan worden. Deze vasculaire hypothese in relatie tot OMD wordt in **hoofdstuk 3.2** uitgewerkt aan de hand van de vaatvoorziening van het netvlies. Dunnere arteriolen, noch dikkere venulen waren geassocieerd met het risico van OMD. Dit bevestigt de huidige opvattingen dat OMD meer ontstaat door veranderde doorbloeding in structuren die onder het netvlies liggen: vaatvlies (chorioidea) en het daaruit gevoede retinale pigment epitheel. Daarom onderzochten we in **hoofdstuk 3.3** of oververziendheid

(hypermetropie), die tot een belemmerde choroidale circulatie zou leiden door een toegenomen stijfheid of dikte van de buitenzijde van het oog, de harde oogrok (sclera), gerelateerd is aan OMD. Inderdaad hadden hypermetrope ogen een verhoogde kans op het ontwikkelen van OMD. In **hoofdstuk 3.4** wordt besproken of retinale vaten een rol spelen in het ontstaan van een andere oogziekte, namelijk primair open-kamerhoek glaucoom. Noch arteriolaire, noch venulaire diameters waren geassocieerd met de kans op dit soort glaucoom. Tevens waren retinale vaatdiameters niet voorspellend voor veranderingen van de oogzenuw of in de breedte van de zenuwvezelrand van deze zenuw.

**Deel IV** richt zich op neurologische aandoeningen. In **hoofdstuk 4.1**, toonden we aan dat grotere venulaire diameters gerelateerd waren aan een hogere kans op beroerte en herseninfarct. In **hoofdstuk 4.2**, worden data gepresenteerd afkomstig van een subgroep van mensen zonder dementie, bij wie een MRI scan is gemaakt. Ook hier vonden we dat grotere diameters van de ader gerelateerd waren aan progressie van afwijkingen in de witte stof van de hersenen, zowel direct rond de hersenventrikels als in de dieper gelegen witte stof. Bovendien, hadden personen met grotere venulen meer kans op nieuwe doorbloedingsstoornissen van de hersenen die resulteerden in kleine infarcten. Omdat vaatveranderingen in de hersenen niet alleen een belangrijke rol spelen in vasculaire dementie, maar ook in de ziekte van Alzheimer, onderzochten wij de associatie tussen retinavaten en dementie in **hoofdstuk 4.3**. Opnieuw vonden we dat grotere venulaire diameters geassocieerd waren met een verhoogd risico op dementie, inclusief de ziekte van Alzheimer en vasculaire dementie. Alle associaties waren onafhankelijk van andere bekende cardiovasculaire risicofactoren. Behalve de retinavaten, onderzochten we in **hoofdstuk 4.4** of andere retina-afwijkingen, zoals die gezien worden in OMD, gerelateerd waren aan de ziekte van Alzheimer. Onze resultaten pleitten ertegen dat OMD een oculaire manifestatie is van de ziekte van Alzheimer of vice versa.

**Deel V** verkent de associaties tussen retinavaten en het risico van aandoeningen elders in het lichaam. In **hoofdstuk 5.1**, bevestigden we dat kleinere arteriolaire diameters samengingen met een verhoogd risico op hoge bloeddruk (hypertensie). Dit suggereert dat gegeneraliseerde arteriolaire vernauwing de ontwikkeling van hypertensie zou voorafgaan. In **hoofdstuk 5.2**, vonden we dat verwijding van de aders, meer dan vernauwing van de slagaders, de belangrijkste verklaring voor een kleiner worden van de AVR vormden. Deze kleinere AVR ging gepaard met een verhoogd risico op een verhoogd nuchtere bloedsuikerspiegel ("impaired fasting glucose") en op het

ontstaan van suikerziekte. In het laatste hoofdstuk van dit deel (**hoofdstuk 5.3**), lieten wij zien dat grotere venulaire diameters gerelateerd waren aan een verhoogd risico op mortaliteit.

Tot slot, worden in **hoofdstuk 6** de methodologie, de belangrijkste bevindingen en suggesties voor verder onderzoek besproken. Mijn uiteindelijke conclusies waren dat venulen niet constant blijven onder verschillende pathologische omstandigheden en dat ze een onafhankelijke rol spelen in het bepalen van de kans op cardiovasculaire en cerebrovasculaire aandoeningen. Het ontrafelen van de mechanismen, die ten grondslag liggen aan venulaire afwijkingen, zou nieuwe inzichten kunnen verschaffen over de ontstaanswijze van ouderdomsziekten.

# *Part VII*

# *Epilogue*

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*"If I have seen further than other men,*
*It is because I stood on the shoulders of giants"*
Isaac Newton (1642-1727)

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*M. K. Ikram*

# List of Publications

1. **M.K. Ikram**, F.J. de Jong, J.R. Vingerling, J.C.M. Witteman, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2004; 45: 2129-2134

2. R. van Leeuwen, **M.K. Ikram**, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong. Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2003; 44: 3771-3777

3. **M.K. Ikram**, R. van Leeuwen, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong. Retinal vessel diameters and the risk of incident age-related macular disease: The Rotterdam Study. *Ophthalmology* 2005; 112: 548-552

4. **M.K. Ikram**, R. van Leeuwen, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong. Relationship between refraction and prevalent as well as incident age-related maculopathy: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2003; 44: 3778-3782

5. **M.K. Ikram**, S. de Voogd, R.C.W. Wolfs, A. Hofman, M.M.B. Breteler, L.D. Hubbard, P.T.V.M. de Jong. Retinal vessel diameters and incident open-angle glaucoma and optic disc changes: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 2005; 46: 1182-1187

6. **M.K. Ikram**, F.J. de Jong, M. Bos, J.R. Vingerling, A. Hofman, P.J. Koudstaal, P.T.V.M. de Jong, M.M.B. Breteler. Retinal vessel diameters and risk of stroke: The Rotterdam Study. *Submitted*

7. **M.K. Ikram**, F.J. de Jong, E.J. van Dijk, N.D. Prins, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Retinal vessel diameters and cerebral small vessel disease: The Rotterdam Scan Study. *Submitted*

8. F.J. de Jong, **M.K. Ikram**, P.J. Koudstaal, J.R. Vingerling, A. Hofman, P.T.V.M. de Jong, M.M.B. Breteler. Retinal arteriolar and venular diameters and risk of dementia: The Rotterdam Study. *Submitted*

9. **M.K. Ikram**, J.R. Vingerling, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Aging macular disease in relation to Alzheimer's disease and brain atrophy on MRI. *Submitted*

10. **M.K. Ikram**, J.R. Vingerling, A. Hofman, J.C.M. Witteman, M.M.B. Breteler, P.T.V.M. de Jong. Retinal vessel diameters and risk of hypertension: The Rotterdam Study. *Submitted*

11. **M.K. Ikram**, J.A.M.J.L. Janssen, A.M.E. Roos, I. Rietveld, A. Hofman, C.M.van Duijn, P.T.V.M. de Jong. Retinal vessel diameters and risk of diabetes mellitus: The Rotterdam Study. *Submitted*

12. **M.K. Ikram**, J. Heeringa, J.R. Vingerling, A. Hofman, M.M.B. Breteler, P.T.V.M. de Jong. Retinal vessel diameters and all-cause mortality: The Rotterdam Study. *To be submitted*

***Other Publications***

13. E. Skenduli-Bala, S. de Voogd, R.C. Wolfs, R. van Leeuwen, **M.K. Ikram**, J.B. Jonas, D. Bakker, A. Hofman, P.T.V.M. de Jong. Causes of incident visual field loss in a general elderly population: the Rotterdam study. *Arch Ophthalmol*. 2005; 123: 233-8.

14. J.H. Kempen, P. Mitchell, K.E. Lee, J.M. Tielsch, A.T. Broman, H.R. Taylor, **M.K. Ikram**, N.G. Congdon, B.J. O'Colmain; Eye Diseases Prevalence Research Group. The prevalence of refractive errors among adults in the United States, Western Europe, and Australia. *Arch Ophthalmol*. 2004; 122: 495-505.

15. **M.K. Ikram**, P.H. Borger, J.J. Assink, J.B. Jonas, A. Hofman, P.T.V.M. de Jong. Comparing ophthalmoscopy, slide viewing, and semi-automated systems in optic disc morphometry. *Ophthalmology*. 2002; 109: 486-93.

16. R.S. Ramrattan, R.C. Wolfs, **K. Ikram**, J.B. Jonas, P.T.V.M. de Jong. Author's reply *Ophthalmology*. 2000; 107: 1218-9.

17. S. de Voogd, **M.K. Ikram**, R.C. Wolfs, A. Hofman, N.M. Jansonius, P.T.V.M. de Jong. Incidence of open-angle glaucoma in a general elderly population. The Rotterdam Study. *Ophthalmology. In press*.

18. S. de Voogd, **M.K. Ikram**, R.C.W. Wolfs, N.M. Jansonius, J.C.M. Witteman, A. Hofman, P.T.V.M. de Jong. Association between diabetes mellitus and incident

open-angle glaucoma. *Submitted*

19. I. Rietveld, **M.K. Ikram**, J.R. Vingerling, A. Hofman, H.A.P. Pols, S.W.J. Lamberts, P.T.V.M. de Jong, C.M. van Duijn, J.A.M.J.L. Janssen. An insulin-like growth factor-I gene polymorphism modifies the risk to develop diabetic retinopathy. *Submitted*

20. **M.K. Ikram**, S. Sabour, A. Hofman, N.M. Jansonius, P.T.V.M. de Jong. Seven-year incidence of refractive errors and astigmatism in the general elderly population: The Rotterdam Study. *To be submitted*

# Curriculum Vitae

Mohammad Kamran Ikram was born on December 27th, 1976 in Rotterdam, the Netherlands. He graduated in July 1995 at the Gymnasium Erasmianum in Rotterdam. In the same year he started his medical studies at the Erasmus University Rotterdam. During his medical studies he participated in the Master of Science program in Clinical Epidemiology. In 1999, he went to Boston, USA to attend the 19th Annual Epidemiology Summer Program and after returning from the USA he completed his Master of Science degree at the Netherlands Institute of Health Sciences. He obtained his medical degree in 2001 (propedeuse and medical degree *cum laude*). In September 2001, he started the work described in this thesis under supervision of prof.dr. P.T.V.M. de Jong and prof.dr. M.M.B. Breteler at the Department of Epidemiology & Biostatistics of the Erasmus Medical Center Rotterdam. From September 2005 onwards he will be working as a resident (AGNIO) at the Department of Neurosurgery (head: prof. dr. C.J.J. Avezaat), Erasmus MC.