Age-related macular disease

Studies on incidence, risk factors, and prognosis

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ISBN 90-73235-217-3

Age-related macular disease. Studies on incidence, risk factors, and prognosis.
PhD thesis, Erasmus University Rotterdam, The Netherlands, 2003
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Cover: Aristotle with a bust of Homer. Oil on canvas, 1653, Rembrandt Harmensz van Rijn (1606-1669). New York, The Metropolitan Museum of Art. It shows the contemplation of Aristotle (or Apelles?) with one hand resting on a gold chain, showing a small portrait of Alexander the Great, while the other hand rest on a bust of the Homer. Note the absence of light in the eyes of Homer, indicating his blindness.

Design and layout by Redmer van Leeuwen
Printed by Optima Grafische Communicatie, Rotterdam.

Age-related macular disease

Studies on incidence, risk factors, and prognosis

Ouderdoms maculadegeneratie

Studies naar incidentie, risicofactoren en prognose

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector Magnificus

Prof.dr.ir. J.H. van Bemmel

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 8 oktober 2003 om 13:45 uur
door

Redmer van Leeuwen

geboren te Groningen.

Promotiecommissie

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".....Daarom kan het belang van zelfkritiek niet genoeg worden benadrukt,
deze dient alle denken te volgen als een schaduw van wantrouwen.
Het zichzelf kunnen hernemen is de kwaliteit der sterken."

Willem Brakman, 2002

Acknowledgment

The work presented in this thesis was conducted at the Department of Epidemiology and Biostatistics of Erasmus Medical Center, Rotterdam. The author gratefully acknowledges the participants, principal investigators, field workers, and general practitioners of the Rotterdam Study. Also, the collaboration with Prof. Usha Chakravarthy (Department of Ophthalmology and Vision Science of The Queen's University of Belfast, Northern Ireland), Prof. Ron Klein, Prof. Barbara Klein, and Sandra Tomany (Department of Ophthalmology and Visual Science of University of Wisconsin-Madison, USA), Prof. Paul Mitchell and Jie Jin Wang (Department of Ophthalmology of University of Sydney, Sydney, Australia), and Prof. Bob Cumming (Department of Public Health and Community Medicine of University of Sydney, Sydney, Australia) is gratefully acknowledged.

The ophthalmic part of the Rotterdam Study is supported by the Optimix Foundation, the Netherlands Organization for Scientific Research, the Netherlands Society for the Prevention of Blindness, Blindenhulp Foundation, Rotterdamse Blindenbelangen Foundation, OOG Foundation, and Topcon Europe BV.

Publication of this thesis is supported by the Department of Epidemiology and Biostatistics of Erasmus Medical Center Rotterdam, the Netherlands Organization for Scientific Research, Stichting voor Ooglijders, Laméris Ootech BV, GlaxoSmithKline BV, Pharmacia BV, ERGRA Low Vision, Alcon Nederland BV, Novartis Ophthalmics BV, MSD BV, en Tramedico BV.

Contents

Abbreviations

APOE	apolipoprotein E
ARM	age-related maculopathy
AMD	age-related macular disease/degeneration
BDES	Beaver Dam Eye Study
BMES	Blue Mountains Eye Study
CI	confidence interval
CInc	cumulative incidence
D	dioptre
DHA	docosahexaenoic acid
iARM	incident age-related maculopathy
IR	incidence rate
HR	hazard rate
HDL	high-density lipoprotein
MUFA	monounsaturated fatty acids
OAG	open angle glaucoma
OMD	ouderdoms maculadegeneratie
OR	odds ratio
pARM	prevalent age-related maculopathy
PUFA	polyunsaturated fatty acids
RPE	retinal pigment epithelium
RR	rate ratio
RS	Rotterdam Study
SD	standard deviation
SE	standard error
SphE	spherical equivalents

Publications and manuscripts based on studies included in this thesis

Chapter 1.2 Van Leeuwen R, Klaver CCW, Vingerling JR, Hofman A, de Jong PTVM. Epidemiology of age-related maculopathy: a review. Eur J Epidemiol 2003, in press.

Chapter 2.1 Van Leeuwen R, Klaver CCW, Vingerling JR, Hofman A, de Jong PTVM. The risk and natural course of age-related maculopathy. Follow-up at 6½ years in the Rotterdam Study. Arch Ophthalmol 2003;121:519-526.

Chapter 2.2 Van Leeuwen R, Chakravarthy U, Vingerling JR, Brussee C, Hooghart AJ, Mulder PG, de Jong PTVM. Grading of age-related maculopathy for epidemiological studies: is digital imaging as good as 35-millimeter film? Ophthalmology 2003;110:1540-1544.

Chapter 3.1 Ikram MK, van Leeuwen R, Vingerling JR, Hofman A, de Jong PTVM. The relationship between refraction and prevalent as well as incident age-related maculopathy: the Rotterdam Study. Invest Ophthalmol Vis Sci 2003, in press.

Chapter 3.2 Van Leeuwen R, Ikram MK, Vingerling JR, Witteman JCM, Hofman A, de Jong PTVM. Blood pressure, atherosclerosis and the incidence of age-related maculopathy: the Rotterdam Study. Invest Ophthalmol Vis Sci 2003, in press.

Chapter 3.3 Van Leeuwen R, Klaver CCW, Vingerling JR, Hofman A, van Duijn CM, Stricker BHCh, de Jong PTVM. Cholesterol and age-related maculopathy: is there a link? Submitted.

Chapter 3.4 Van Leeuwen R, Klaver CCW, Vingerling JR, Witteman JCM, Hofman A, de Jong PTVM. Dietary intake of antioxidants and risk of age-related maculopathy in the Rotterdam Study: a prospective population-based study. Submitted.

Chapter 3.5 Van Leeuwen R, Vingerling JR, Witteman JCM, Hofman A, de Jong PTVM. Fat for the fundus: dietary fat intake and age-related maculopathy. Submitted.

Chapter 3.6 Van Leeuwen R, Tomany SC, Wang JJ, Klein R, Mitchell P, Hofman A, Klein BEK, Vingerling JR, Cumming RG, de Jong PTVM. Is medication use associated with the incidence of early age-related maculopathy? Pooled findings from three continents. Submitted.

Chapter 3.7 Van Leeuwen R, Vingerling JR, Hofman A, de Jong PTVM, Stricker BHCh. Cholesterol lowering medication and risk of age-related maculopathy: prospective cohort study with cumulative exposure measurement. BMJ 2003;326:255-256.

Chapter 4 Borger PH, van Leeuwen R, Hulsman CAA, Wolfs RCW, van der Kuip DA, Hofman A, de Jong PTVM. Is there a direct association between age-related eye diseases and mortality? The Rotterdam Study. Ophthalmology 2003;110:1292-1296.

1
Introduction

1.1 Age-related macular disease

Age-related macular disease (AMD) is a new name, recently coined by Bird,[25] for a progressive and degenerative disease in elderly persons affecting the macula lutea. Dysfunction of this part of the retina, and especially its centre, the fovea, results in the inability to read, recognize faces, drive, and move freely.

Bird proposed to substitute the word *disease* for *degeneration*, probably due to the pejorative ring of the latter and because the word *degeneration* does not cover all pathological processes thought to play a role in AMD. According to the current nomenclature of the International AMD Epidemiological Study Group, all early and late signs of AMD are called age-related maculopathy (ARM), while age-related macular degeneration indicates the two late stages of ARM.[24] These are the dry type, also called geographic atrophy, and the wet type, also called neovascular or disciform AMD. The wet type often leads to rapid loss of central vision while this may take years for the dry type.

The oldest image of disciform AMD, as far as we know, is from 1875 by Pagenstecher and Genth, while Haab was in 1885 the first to describe the dry type of AMD that he called senile macular degeneration. I decided to follow Bird's example in the title of this thesis and used the word *disease* instead of *degeneration*. However, the reader will find in the remainder of this book the term ARM and AMD in the conventional sense, because most articles included in the thesis were written at a time that we still used ARM as the common denominator for this disease.

As suggested by its name, AMD is strongly related to age. More specifically, AMD is very rare in persons under 50 years of age, while its prevalence steeply increases after the age of 70. Personally, I would prefer to replace the preposition *age-related* by *ageing*. The latter term refers to the process of getting old, while *age-related* does not make a distinction between young or old age. This is still open for debate. Because of the rapid increase in the proportion of elderly people in the population, both in developed and developing countries, the prevalence of AMD is expected to rise strongly. The impact of AMD on public health is likely to increase proportionally, given the paucity of preventive and therapeutic interventions for the majority of patients. Numerous studies, both in basic and clinical science, have provided suggestions for one or more causal mechanisms, but definite evidence has not been presented yet.

Hypotheses on the cause of AMD include vascular insufficiency due to atherosclerosis, cumulative exposure to oxidative stress, or the destructing effect of inflammatory processes.

The research described in this thesis is a further attempt to elucidate the aetiology of AMD. In this case, an epidemiological approach is employed. Epidemiology is the science of the occurrence of disease. It investigates disease frequency and factors that determine this frequency. A basic assumption of epidemiological research is that factors associated with disease frequency give us insight into the aetiology of that disease. By carefully analysing the association between risk factor and disease, in particular through adjustments for appropriate confounders, one can learn about causal pathways. If a risk factor is causally linked to a disease, and if this factor can be modified, its identification offers the potential of prevention.

Both frequency of AMD and its risk factors are investigated in this doctoral thesis. First, a review of previous research on the epidemiology of AMD is presented and, subsequently, the research objectives are formulated.

1.2 Epidemiology of age-related maculopathy: a review

Age-related maculopathy (ARM) is a degenerative disease of the retina and the leading cause of incurable blindness and visual impairment among older people in industrialized countries. The aetiology of ARM is still unknown, despite intensive research on many fronts. In this paper, we provide a review of the epidemiology of ARM. The most prominent findings were an exponential increase in frequency with age, a significant familial and genetic component, and a strong association with smoking. Other risk factors that were found less consistently were atherosclerosis, low intake of antioxidant nutrients, and cataract extraction. Future studies, both observational and experimental, will hopefully identify more risk factors that are amenable to prevention. ***Eur J Epidemiol 2003, in press.***

In this chapter, a review will be provided of the epidemiology of ARM. The literature on the frequency of ARM will be summarized, as well as the main findings on genetic, ocular, systemic, environmental, and dietary risk factors. Ambati et al have recently published a review of the aetiology, pathogenesis, and therapeutic strategies of ARM.[15] This review starts with a short discussion of the diagnosis of ARM.

DIAGNOSIS

The diagnosis of ARM can be made by visual inspection of the retina (funduscopy or fundus photography). Fluorescein or indocyanine angiography may give additional information, but is usually only necessary for sub-classification. A number of investigators of large cohort studies have established a protocol for the diagnostic work-up of ARM for epidemiological purposes.[24] In this protocol, standards for fundus photography, grading of transparencies, and classification of signs of ARM were proposed. Only studies using this or a similar protocol for ARM diagnosis, including photo recording and reading, were considered for this review.

An early and a late stage of ARM can be distinguished. Late ARM is also called age-related macular degeneration (AMD) and is responsible for loss in visual acuity, in contrast to the early pre-symptomatic stage. The main

characteristic of ARM is the presence of drusen, yellow deposits under the retinal pigment epithelium (RPE), and pigment changes, both hyper- and hypo-pigmentation. Drusen may vary in size, structure, and shape, but they tend to grow, thereby leading to an increased risk of AMD. AMD can be present in two distinct forms, atrophic or neovascular AMD. Atrophic AMD, also known as geographic atrophy or dry AMD, is characterized by well-defined areas of atrophy of the RPE and underlying choriocapillaris. With neovascular AMD, also called exudative or wet AMD, new blood vessels sprout from the choriocapillaris resulting in a serous or hemorrhagic detachment of the RPE and subsequent periretinal scarring in the macular area.

Drusen must be differentiated from retinal lesions due to other conditions, for example hard exudates, cotton wool spots, and flecks accompanying fundus flavimaculatus or fundus albipunctatus. Pigment changes of the retina can also be seen as part of other abnormal processes, such as chorioretinitis, long-standing cystoid macular oedema, trauma, or laser photocoagulation. Neovascular AMD may resemble myopic macular degeneration, chorioretinitis, pseudoxanthoma elasticum, Paget's disease, presumed ocular histoplasmosis syndrome, toxoplasmosis, central areolar choroidal sclerosis, and other more rare pathological processes. So, the diagnosis of ARM will always be one by exclusion of other ocular diseases.

FREQUENCY

Prevalence and incidence

Population-based studies on the prevalence of ARM were conducted in different parts in the world, but diagnostic procedures and definitions varied widely. This is particular true when the early signs of ARM were included, making comparisons between populations based on these data difficult. The diagnosis of atrophic or neovascular AMD shows less variation. Prevalence estimates from all studies show an exponential increase after the age of 70.[29, 90, 108, 142, 210] A reasonable overall estimate of the prevalence of AMD in persons aged 65-74 years is 1%, increasing to 5% in persons aged 75-84 years, and 13% in persons 85 years and older. The prevalence of early ARM in the age-category 65-74 years is 15%, in the age-category 75-84 years 25%, and in persons 85 years and older 30%.[29, 90, 108, 142, 210]

Four population-based studies have reported on the incidence of ARM.[30, 98, 116, 145] The 5-year cumulative incidence of late ARM in the US Beaver Dam Eye Study was 1.3% for subjects between 65 and 74 years, and 5.4% for subjects aged 75 and older, while these frequencies were 16%, and 23% for early ARM.[116] The same figures from the Australian Blue Mountains Eye Study were rather similar.[145] The incidence in the European Rotterdam Study appeared to be lower, with 5-year incidence estimates of late ARM of 0.6% for subjects aged 65-74 years and 2.8% for persons aged 75 and older, while these age-specific incidences of early ARM were 7% and 18%, respectively.[200] Although these comparisons were hampered by differences in

incidence calculation, the lower incidence estimates are in line with the prevalence findings.

Geographic region

Comparisons in ARM frequency between different geographic regions are difficult due to the many genetic and environmental factors involved. The aforementioned cohort studies from the United States, Netherlands, and Australia, that used similar methods and designs, and included homogenous populations of European descent, investigated prevalence differences.[184] Compared with the Dutch population, the American and Australian study found 1.5 and 2.2 times higher prevalence of AMD, respectively. This difference could not be explained by age or smoking.

Gender

A recent meta-analysis of a large number of prevalence studies showed that women are at a slightly higher risk of any type of ARM, especially at age 75 years and older.[54] However, since women live longer and ARM is strongly related to age, insufficient adjustment for age could be an explanation for this finding.

Ethnicity

Previously discussed frequency estimates were based on white, Caucasian populations. The clinical impression has been that blindness due to ARM is rare in blacks.[87] A few studies have specifically addressed this issue. A study of a black population reported a prevalence of early ARM that was comparable with that among whites, but the frequency of neovascular ARM was much lower.[165] However, since the study cohort did not include other ethnicities for direct comparison, other factors could be accountable. A recent study in a biracial population found indeed that early signs of ARM are frequent in blacks, adjusted for age.[126] A review of the ethnic differences in ARM prevalence suggested that early ARM is equally prevalent among blacks and whites, while late ARM may be more frequent in the latter group.[121] The genetic susceptibility may explain part of the difference in racial disparities, but other factors, such as choroidal melanin concentration may play a role as well. However, the previously mentioned review also concluded that there are insufficient data for a final conclusion on this issue.

DETERMINANTS

Family history and genetic factors

It is now widely acknowledged that the molecular defects leading to ARM are partly inherited.[46] Twin studies, a segregation study, and several familial aggregation studies have all provided evidence for familial occurrence of the trait.[68, 71, 95, 104, 172] A Dutch familial aggregation study based on 222 probands derived from the Rotterdam Study showed that first degree relatives of subjects with late ARM have a 4.2 times increased lifetime risk to develop a similar

stage of ARM.[95] This study showed that relatives of cases have an earlier onset of all features of ARM, and calculated that approximately one fourth of all ARM occurring in a general population is determined by a genetic risk factor. An English twin study compared the occurrence of ARM in 226 monozygotes and 280 dizygotes and calculated heritability scores of ARM.[68] The concordance for ARM in monozygote twins was 0.37 compared with 0.19 in dizygote twins; the heritability of ARM was estimated at 45%.

The candidate gene approach as well as linkage analysis are the main tools in genetic research to identify disease-associated genes. Both have been employed in the genetic dissection of ARM. The presence of one variation in the ABCA4 gene, which causes autosomal recessive Stargardt disease when two alleles are mutated, has been associated with ARM. This gene is specifically expressed in photoreceptor outer segments and appears to function as a flippase for N-retinylidene-PE, an essential component of the visual cycle. The initial study by Allikmets et al. raised some controversy mainly regarding the statistical evidence of the association.[12] In a subsequent large consortium study the association was replicated in 1218 cases and 1258 controls for the two most common ABCA4 alleles with more profound statistical significance.[13] Although several other studies with smaller sample size have not been able to confirm the finding,[191] studies in mice have increased the plausibility of a role of ABCA4 in ARM by showing that absence of one functional copy of the ABCA4 gene leads to retinal degeneration.[219]

Another candidate gene studied in ARM is the cholesterol transporter gene apolipoprotein E (APOE). This gene has an ancestral allele, E3, and 2 common variants with opposing biochemical characteristics, i.e., E2 and E4. Klaver et al. and Souied et al. initially showed that the E4 allele was significantly less frequent in AMD cases.[94, 188] Many studies,[167, 175] although not all,[153, 169] have since then replicated the finding. Animal studies have revealed that APOE deficiency leads to retinal pathology especially in a cholesterol-rich environment. APOE knockout mice fed on a cholesterol-rich diet displayed a lower number of cell nuclei in inner and outer nuclear layer, a thicker Bruch's membrane, and a disorganized elastic lamina.[151] Mice variants carrying the three human APOE isoforms expressed retinal pathology mainly when fed on a high fat diet.[132] Surprisingly, the APOE E4 mice variant also displayed retinal pathology, contradicting a protective effect. In summary, there is now ample evidence that subjects carrying the APOE E4 allele have a decreased risk of AMD, and that variants of the APOE gene disturb normal retinal physiology in a cholesterol-dependent way. The nature of the direction of the epidemiological association is as yet not explained.

Two small Japanese studies performed association analyses on genes involved in lipoprotein oxidation and enzymatic oxidation processes.[84, 93] A skewed distribution between subjects with neovascular AMD and healthy controls was found for two alleles in the paraoxonase gene, and one allele in the manganese superoxide dismutase gene. These findings need confirmation,

but they are interesting since they shed new light on the role of oxidative stress in the pathogenesis of ARM.

Genome wide screens and linkage analyses in cohorts of sib ships and small families have provided a variety of susceptibility loci with mostly low Lodd scores.[166, 216, 217] The latter is likely to be the result of the large range of genetic heterogeneity of minor disease genes. A large family with atrophic AMD described by Klein et al showed statistically significant linkage to chromosome 1q25-31, and recently the authors provided evidence that the FIBL-6 gene may be the disease-associated gene in this affected family.[168] This gene is a member of the immunoglobulin super-family and appears to be involved in stabilization and organization of extra-cellular matrix. Replication of gene association and functional studies are now needed to evaluate the importance of this gene for ARM.

Recent availability of the human genome sequence has allowed for a dramatic acceleration in disease gene discovery. It is our expectation that many more genes involved in ARM will become known within the next decade.

Ocular factors

Dark coloured irises, compared with blue ones, are thought to correlate with more densely pigmented choroid, and thereby protect the retina better against light damage.[231] Initially, two case-control studies reported a protective effect of dark irises for ARM,[83, 218] but this was not confirmed in later studies.[3, 206, 221] Holz et al. reported no association with light iris colour, but noted that self-reported decrease of iris pigmentation during life was associated with ARM.[75] Among the population-based studies, only the Blue Mountains Eye Study found an increased odds ratio of blue eyes for ARM.[144] The Beaver Dam Eye Study did not find an association with incidence or progression of ARM.[118] The inconsistency of data does not suggest a strong effect.

Maltzman et al. were the first to suggest that refractive error was associated with AMD.[133] More specifically, hyperopia would be associated with an increased risk of AMD. Later, four case-control studies confirmed this finding.[3, 83, 163, 218] The Blue Mountains Eye Study found a weak positive relation between moderate hyperopia and early ARM, but not with AMD.[214] In contrast, the Beaver Dam Eye Study reported a weak inverse association between hyperopia and incident early ARM.[118] A concern in interpreting this association is the difficulty in distinguishing neovascular AMD and neovascularization due to severe myopia. This problem may explain the positive association between hyperopia and neovascular AMD, but not with early ARM.

There are suggestions for a relation between cataract and ARM. In the Beaver Dam Eye Study, an association between nuclear cataract and prevalent early ARM was observed at baseline.[112] Further evidence for a positive relation was provided by the 10-year incidence data from this study, which indicated a 30-40% risk increase of early ARM for subjects with cataract at baseline.[125] No relation was found with AMD.

An increased risk of neovascular AMD after cataract extraction was first suggested by a histopathological study.[198] Later, this finding was confirmed in a clinic-based setting.[155] The Beaver Dam Eye Study convincingly showed a long-term negative effect on the outcome of ARM after cataract extraction.[125] Subjects who had undergone cataract surgery at baseline developed neovascular as well as atrophic AMD at a 3-4 times increased rate. The finding is of great clinical importance, for it suggests a conservative policy towards subjects with early ARM and cataract. Possible explanations for the association, apart from a detection bias, are mechanical damage to Bruch's membrane, light injury during or after the operation, or an inflammatory response.

Systemic conditions

A possible explanation for the higher prevalence of ARM among women, if true, may be the loss of a protective effect of estrogens against atherosclerosis in postmenopausal women. The first indication for such a relation came from a large case-control study.[3] The Rotterdam Study found that women with an early menopause due to surgical removal of their ovaries had a higher risk of AMD.[211] The Blue Mountains Eye Study observed that women with early ARM reported less years from menarche to menopause.[180] A beneficial role of endogenous oestrogen could not be confirmed in Beaver Dam, neither with prevalent nor with incident ARM.[101, 103] Data on the impact of hormone-replacement therapy have been inconclusive so far.[3, 101, 103]

One hypothesis on the pathogenesis of ARM is that vascular disease affects the choriocapillaris underlying the retina. This would result in an impaired supply of nutrients and decreased outflow of waste products. The issue was examined either by investigating a history of cardiovascular disease or its risk factors, or by direct measurement of systemic atherosclerosis. Two caveats may underlie the overall inconsistent findings. Vascular risk factors may have their effect well before the onset of ARM, or systemic vascular determinants may not reflect the vascular status of the eye very well. Conflicting reports have been published about the association between ARM and a history of cardiovascular disease; some studies have found a positive association,[64, 83] whereas most did not.[3, 7, 82, 140] Self-reported history of cardiovascular disease, however, is potentially biased by misclassification, making it more difficult to detect a real association. The data on an association between hypertension and overall ARM are generally inconclusive and weak.[189] However, the data on systemic hypertension and risk of neovascular AMD appear to be more concordant. Two recent case-control studies found a significant association between hypertension, or use of antihypertensive medication, and neovascular but not atrophic AMD.[7, 82] This was substantiated by two studies, which confirmed an association between uncontrolled hypertension and risk of neovascular AMD.[5, 115]

In the Rotterdam Study, Vingerling et al. found that plaques in the carotid artery were associated with prevalent AMD.[208] This finding was

confirmed in the long-term follow-up phase on this study. Both indirect measures of atherosclerosis such as pulse pressure and ankle-arm index, and direct measurements of carotid wall thickness and carotid plaques, were associated with an increased risk of incident ARM.[203] The Beaver Dam Eye Study found that higher pulse pressure was associated with an increased 5-year incidence of neovascular ARM.[115] Of interest in this relation is the histopathologic finding that drusen consist of proteins common to atherosclerosis-related extracellular deposits.[148]

Hyperglycaemia may be associated with ARM through its relation with atherosclerosis, or it may directly cause retinal capillary non-perfusion.[34] A number of studies investigated the relation between diabetes and ARM, but none found an association.[3, 83, 115, 109] The effect of hyperglycaemia, if any, is likely to be small.

Histopathological studies have shown that fatty acids and cholesterol accumulate with age in Bruch's membrane.[76] This deposition may interfere with the normal function of the RPE and promote ARM development. Also, serum lipids promote the process of atherosclerosis, which is implicated as a risk factor of ARM. Of the studies that have investigated the relation between total cholesterol levels and ARM, only one large case-control study found a positive association,[3] while most others did not.[44, 184, 181] Interestingly, many studies reported a positive association between high-density lipoprotein cholesterol and neovascular AMD or early ARM.[50, 82, 111, 122] No explanation for this relationship has been provided yet.

Environmental exposures

An increased risk of ARM in smokers was first suggested by Paetkau et al.[152] Since then, most studies have confirmed the positive association between smoking and ARM.[3, 83, 140, 178, 212] The association was particularly strong in neovascular AMD,[212] and seemed to have a strong dose-response relationship. Interestingly, two studies found that the longer the non-smoking period after cessation, the lower the risk of former smokers.[47, 212] In a pooled analysis of three population-based studies, smoking was the only consistent risk factor in all participating cohorts.[184] Recent reports on incident cases confirmed the strong associations between smoking and ARM, supporting a causal relation. Seddon et al. evaluated the relation between cigarette smoking and self-reported incident AMD in the large Nurses' Health Study and found a relative risk of 2.4 for women smoking over 25 cigarettes per day.[171] Christen et al. evaluated this relationship in the Physicians' Health Study and found a relative risk of 2.5 for men smoking over 20 cigarettes per day.[38] In the 5-year follow up of the Beaver Dam Eye Study, Klein et al. found an increased risk of incident early signs of ARM.[119] Several mechanisms could play a role, but none have been proven. It is plausible that by reducing serum antioxidants smoking decreases retinal levels of antioxidant enzymes as well. These enzymes protect the macula against oxygen radicals formed during light exposure.[19] Several other pathways could be involved, including alteration of the choroidal blood

flow.[187] The association is important since smoking is still very common and amenable to prevention. The data justify ophthalmologists to advise patients with early signs of ARM to stop smoking.

The use of alcohol was studied as a potential risk factor since it may cause oxidative stress and may interfere with atherosclerosis development. Most studies found no association.[11, 35, 177] Only the Beaver Dam Eye Study reported that beer drinking was associated with an increased risk of ARM, both in a cross-sectional and prospective analysis.[124, 147, 158] The lack of confirmation of this relation by other studies suggests that alcohol is not a strong determinant of ARM.

The damaging effect of long-term light exposure on the photoreceptors and RPE has been reported in several experimental studies and may play a role in the pathogenesis of ARM.[231] However, the difficult quantification of light exposure has limited the ability of epidemiological studies to support this hypothesis. Most studies failed to find a significant association.[83] One study among outdoor workers (fishermen) was specifically designed to address this issue and light exposure was measured extensively and objectively.[159, 221] No association between UV-A or UV-B exposure and ARM was observed, but exposure to blue light did appear to be associated with neovascular AMD.[194] Cruickshanks et al. found a positive association between self-reported time spent outdoors, as well as an inverse association between the use of hats or sunglasses, and prevalent early ARM and neovascular AMD.[43] Recently, this group has confirmed the association between leisure time spent outdoors before the age of 40 and early ARM in the follow-up of the Beaver Dam cohort.[45]

Diet

The strong rise with age, the deleterious influence of smoking, and the potentially harmful effect of cumulative light exposure as described above, all suggest an important role for oxidative stress in the pathogenesis of ARM. The combination of a high concentration of oxygen and polyunsaturated fatty acids, and intense exposure to light, render the retina especially susceptible to the production of oxygen radicals.[19] As a defence, the retina contains high levels of antioxidant enzymes, like superoxide dismutase and glutathione peroxidase. These enzymes need nutritional cofactors, like copper and zinc, which are found in high concentrations in ocular tissues. Other protection may come from vitamins and certain carotenoids, which are oxygen radical scavengers. This theory raises the question whether dietary intake and serum levels of antioxidant vitamins are associated with ARM.[190] Evidence for such an association has first emerged from basic research.[33] Later, the question was investigated in observational as well as intervention studies.

In 1994, West et al. found low serum levels of α-tocopherol (vitamin E) in subjects with ARM.[220] Other studies confirmed an inverse relation between ARM and vitamin E, either with dietary intake, serum levels, or supplement use.[3, 49, 136] However, negative findings regarding vitamin E have also been reported.[4, 134, 179] Vitamin C is another important antioxidant vitamin and

experimental studies have suggested a protective effect against retinal light damage. In contrast, none of the studies in humans has been able to show a relation between vitamin C and risk of ARM.[3, 170, 220] Carotenoids are potent scavengers of oxygen radicals, and the macula contains large amounts of carotenoids, in particular lutein and zeaxanthin. Epidemiological studies investigating the relation between carotenoids and ARM have produced some strong indications for a protective effect, but results were not uniform. A large case-control study reported a 40% reduced risk of neovascular AMD with a high dietary intake of lutein and zeaxanthin.[170] In the same patient population, an inverse association with similar magnitude was found with serum levels of carotenoids.[4] A non-significant inverse association was reported in two smaller studies.[164, 220] Subsequent case-control [134, 179] and cohort studies [56, 136, 182] did not find a protective effect of carotenoids. In the Rotterdam Study the amount of macular pigment, determined by fundus reflectometry was similar in cases with and without early ARM.[22] A trace element that has attracted much attention is zinc, as it is a cofactor for many antioxidant enzymes and found in high concentrations in the retina. Some studies did indeed find an inverse association between zinc intake and ARM,[136] while others did not.[37, 56, 182, 205] In summary, observational studies did not provide unequivocal results on the association between antioxidant nutrients and ARM.

Trials were initiated to evaluate whether antioxidant intake can delay the onset of ARM. In a small, randomised, placebo-controlled trial among 151 subjects in 1988, a beneficial effect of zinc on the natural course of ARM was suggested,[149] but this result could not be confirmed by others.[39, 192] Recently, however, convincing evidence for a protective effect of supplement use came from a large placebo-controlled clinical trial.[8] Subjects with early stages of ARM were randomised to take high-dose supplements of vitamins C and E, β-carotene and zinc. This trial showed a reduction in the 6-year risk of progression to end-stage ARM from 31% to 23%. A smaller trial of vitamin E supplementation did not find any effect on ARM progression after four years of follow-up.[195]

The Beaver Dam Eye Study found that subjects consuming large amounts of saturated fat and cholesterol had an increased risk of prevalent early ARM.[135] The Blue Mountain Eye Study found an association in the same direction for cholesterol and AMD, although the results were not statistically significant.[183] This study also found a protective effect of frequent consumption of fish. Seddon et al. reported an increased intake of vegetable fat, mono- and polyunsaturated fats, and linoleic acid in subjects with AMD.[173] In subjects with low linoleic acid, the risk of AMD was reduced for the intake of fish and omega-three fatty acids. These results indicate that consumption of certain types of fat rather than total fat intake may influence the risk of ARM.

Comorbidity

A few studies have investigated the relation between ARM and other age-related diseases. Degeneration in the eye may have a common pathway with

other neuro-degenerative disorders. Klein et al. found a significant relation between AMD and hearing loss.[117] This association was independent of age and other possible confounders. Some striking similarities in the pathogenesis of Alzheimer's disease and ARM prompted Klaver et al. to study this association within the Rotterdam Study.[97] Subjects with AMD had an increased risk of developing Alzheimer's disease, but this relation seemed completely determined by shared risk factors, smoking and atherosclerosis.

CONCLUSION

ARM is a major cause of severe visual impairment in industrialized societies. The predicted doubling of the elderly population in the next two decades will increase its public health importance significantly. Numerous epidemiological studies have provided a wealth of data to elucidate the aetiology of ARM. The most prominent findings were an exponential increase in frequency with age, a significant familial and genetic component, and a strong association with smoking. Other risk factors that were found less consistently were atherosclerosis, low intake of antioxidant nutrients, and cataract extraction. Well-designed epidemiological studies, observational and experimental, as well as genetic-epidemiological studies will enhance our knowledge of the aetiology of ARM. Hopefully, these studies will identify risk factors that can be modified and thereby enable the prevention of this blinding disease.

1.3 Objective and outline

The objective of the research presented in this thesis is twofold. The first objective is to determine the incidence of age-related maculopathy (ARM) in the general population. With these incidence data, the risk of an individual to develop the blinding end-stage of ARM, based on age, gender, and the presence of early ARM fundus signs, can be estimated. An additional aim of this study is to describe and quantify the natural course of ARM. The second objective is to identify risk factors of ARM. In the studies described in this thesis, a number of ocular, systemic, and exogenous factors are analysed, without denying the importance of genetic determinants of ARM.

Most studies are performed with data from the Rotterdam Study, a population-based prospective cohort study in Rotterdam, the Netherlands. The incidence of ARM is determined after the second follow-up examination with an average period between baseline and follow-up of six and a half years.

In Chapter 2.1, the incidence and natural course of ARM are presented. In Chapter 2.2, the validity of digital fundus photography for the grading of ARM in epidemiological studies is evaluated. Chapter 3.1 describes a study of refractive error and ARM incidence. Cardiovascular risk factors, including blood pressure, atherosclerosis, and serum cholesterol in relation to ARM are examined in Chapters 3.2 and 3.3. In Chapters 3.4 and 3.5, the association of dietary intake of antioxidant nutrients and fat with the risk of ARM is investigated. Chapters 3.6 to 3.7 describe studies examining the possible effect of medication use on the risk of ARM. Whether ARM is related to survival is analysed in Chapter 4. Finally, in Chapter 5, the methodology of the studies, the main findings and their clinical relevance, suggestions for future epidemiological research, and final conclusions are discussed.

2
Incidence

2.1 The risk and natural course of age-related maculopathy: follow-up at 6½ years in the Rotterdam Study

Objective: *To evaluate the natural course of age-related maculopathy (ARM), and to assess the incidence and absolute risk of its final stage, age-related macular degeneration (AMD).* ***Methods:*** *In a population-based prospective cohort study of 6418 persons 55 years and older, we studied the incidence and natural course of ARM. Subjects underwent identical examinations, including stereoscopic fundus photography, at baseline and at 2 and 6½ years' follow-up. Age-related maculopathy was graded according to the International Classification and Grading System for ARM and AMD, and stratified into five exclusive stages. Incidence was expressed in rates and five-year absolute risks.* ***Results:*** *At follow-up 47 new cases of AMD were identified, with a ratio of neovascular-atrophic AMD of 1.4:1. The 5-year risk of AMD increased with more severe stages to 28.0% for subjects 55 years and older with indistinct drusen and pigment irregularities (stage 3). Age, but not gender, independently increased this risk to a maximum of 42.0% for subjects with stage 3 ARM who were 80 years and older. Individual ARM fundus signs that predicted best the development of AMD were 10 or more large drusen (≥125 μm) and 10% or more of the grid area covered by drusen. Subjects who developed atrophic AMD showed no significant (*P*=.25) differences in baseline fundus signs and natural course compared with subjects who developed neovascular AMD.* ***Conclusions:*** *We provided the absolute risk of AMD as a function of age and early ARM fundus signs, and showed that both are prominent independent risk factors. The progression of ARM stages follows, after the appearance of the first soft drusen, a distinct course at a gradual pace that accelerates with increasing age.* ***Arch Ophthalmol 2003;121:519-526****.*

Since the first epidemiological report on age-related maculopathy (ARM) in 1977,[91] its high prevalence among individuals 65 years and older in Western societies has been well documented.[120, 108, 142, 209] The reported prevalences in those aged 65 to 75 years range from 9%[142] to 25%,[108] depending on the definition of ARM, geographic location and ethnicity of the population. In contrast to prevalence, few data are available on the incidence

and natural course of this disease. Most studies on ARM frequency either were cross-sectional or included patients with uniocular end-stage disease who were recruited from clinics. With these designs, it was not possible to examine the rate at which early stages of ARM develop and the speed with which they progress to end stage disease, also called age-related macular degeneration (AMD).

In an earlier report, the initial 2-year incidence rates of AMD were provided and an ARM staging system to stratify the clinical severity of ARM was proposed.[98] Since then, we have prolonged the study period and added significantly to our number of incident ARM outcomes. In the present article, we provide the results from 6½ years of follow-up with the following objectives: to validate our previous AMD incidence estimates, to determine the long-term progression and natural course of ARM, and to assess the absolute risk of AMD as a function of age and the presence and type of early ARM fundus signs.

METHODS

Study population

Information on the identification and description of the baseline study population has appeared in previous reports.[74, 209] Briefly, the Rotterdam Study is a population-based prospective cohort study of all inhabitants 55 years and older of a suburb of Rotterdam. Common cardiovascular, locomotor, neurological, and ophthalmologic diseases of elderly persons are investigated. The medical ethics committee of Erasmus Medical Centre Rotterdam approved of the study protocol, and a written informed consent was obtained from all participants. Baseline interviews and examinations were performed from July 1989 to September 1993, followed by a first follow-up examination from September 1993 to December 1995. A second follow-up screening took place from March 1997 to December 1999.

Of the initial cohort of 10,275 eligible individuals, 7983 (77.7%) participated in the baseline interview of the Rotterdam Study.[209] 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. At baseline, gradable fundus transparencies were available for 6418 participants. Persons with prevalent atrophic or neovascular AMD (n = 106) were excluded from the assessment of AMD incidence, resulting in a cohort of 6312 subjects at risk for incident AMD. Prevalent early ARM was present in 476 (7.5%) of the participants, leaving a cohort of 5836 subjects at risk for incident early ARM. Data for all eligible subjects who participated in at least 1 follow-up examination were entered into the incidence analyses.

Diagnosis of age-related maculopathy

In addition to a standard eye examination, stereoscopic 35° colour photographs were taken centred on the fovea (Topcon TRV-50VT fundus camera, Topcon

Corporation, Tokyo, Japan). The fundus transparencies were graded with 12.5x magnification according to the International Classification and Grading System for ARM and AMD.[24] In this system, all ARM features within a standard grid (diameter 6000μm) around the fovea are recorded. The fundus signs that were graded included the following: number of drusen (0, <10, 10-19, and ≥20, both within and outside the grid) for each of these sizes (<63, ≥63 to <125, and ≥125 μm), largest and most frequent drusen size (<63, <125, <175, or ≥175 μm, or reticular), confluence of drusen (none, <10%, <50%, or ≥50%), most severe drusen type (hard; soft, distinct, and <125 μm; soft, distinct, and ≥125 μm; soft indistinct; or reticular), grid area occupied by drusen (<1%, <10%, <25%, <50%, or ≥50%, for the central, inner, and outer circles), increased pigmentation (none or <125, <175, or ≥175 μm) and hypopigmentation (none, <175 μm, <5x175 μm, less than central circle, more than central circle) of the retinal pigment epithelium (RPE), atrophic AMD, and neovascular AMD.

Graders first graded the follow-up transparencies after which they 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. Graders were trained according to the Wisconsin ARM grading system. Consensus sessions and between-grader comparisons were performed regularly. Weighted kappa values ranged from 0.60 for hard drusen <63 μm to 0.88 for drusen area. All photographs with possible AMD and all uncertain diagnoses were adjudicated by three of us (C.C.W.K., J.R.V., P.T.V.M.d.J.). In addition, the principal investigators of the Beaver Dam Eye Study and the Blue Mountains Eye Study adjudicated all transparencies of incident AMD.

Definitions

Atrophic AMD was defined as any sharply demarcated round or oval area of apparent absence of the RPE, larger than 175μm, with visible choroidal vessels, and no neovascular AMD. Neovascular AMD was defined as the presence of a serous or a hemorrhagic RPE detachment or a subretinal neovascular membrane or a subretinal hemorrhage, or a periretinal fibrous scar. Lesions that were considered to be the result of generalized disease, such as diabetic retinopathy, or chorioretinitis, high myopia, trauma, congenital diseases, or photocoagulation for reasons other than for neovascular AMD, were excluded from ARM grading. We defined early ARM as the presence of either soft distinct drusen ≥63 μm with hyperpigmentation and/or hypopigmentation of the RPE or soft indistinct or reticular drusen with or without pigment irregularities. To study the progression of ARM, and to enhance the clinical application, we stratified the range of ARM fundus signs into five mutually exclusive stages.[98] Definitions and photographic examples of these stages are given in Table 2.1.1 and Figure 2.1.1, respectively. Stages 2 and 3 correspond with early ARM; stage 4 is equal to AMD.

The incidence of early ARM and of AMD was defined as the absence of this diagnosis in both eyes at baseline and the presence of the diagnosis in at least one eye at follow-up. For the risk analysis of individual fundus signs, also

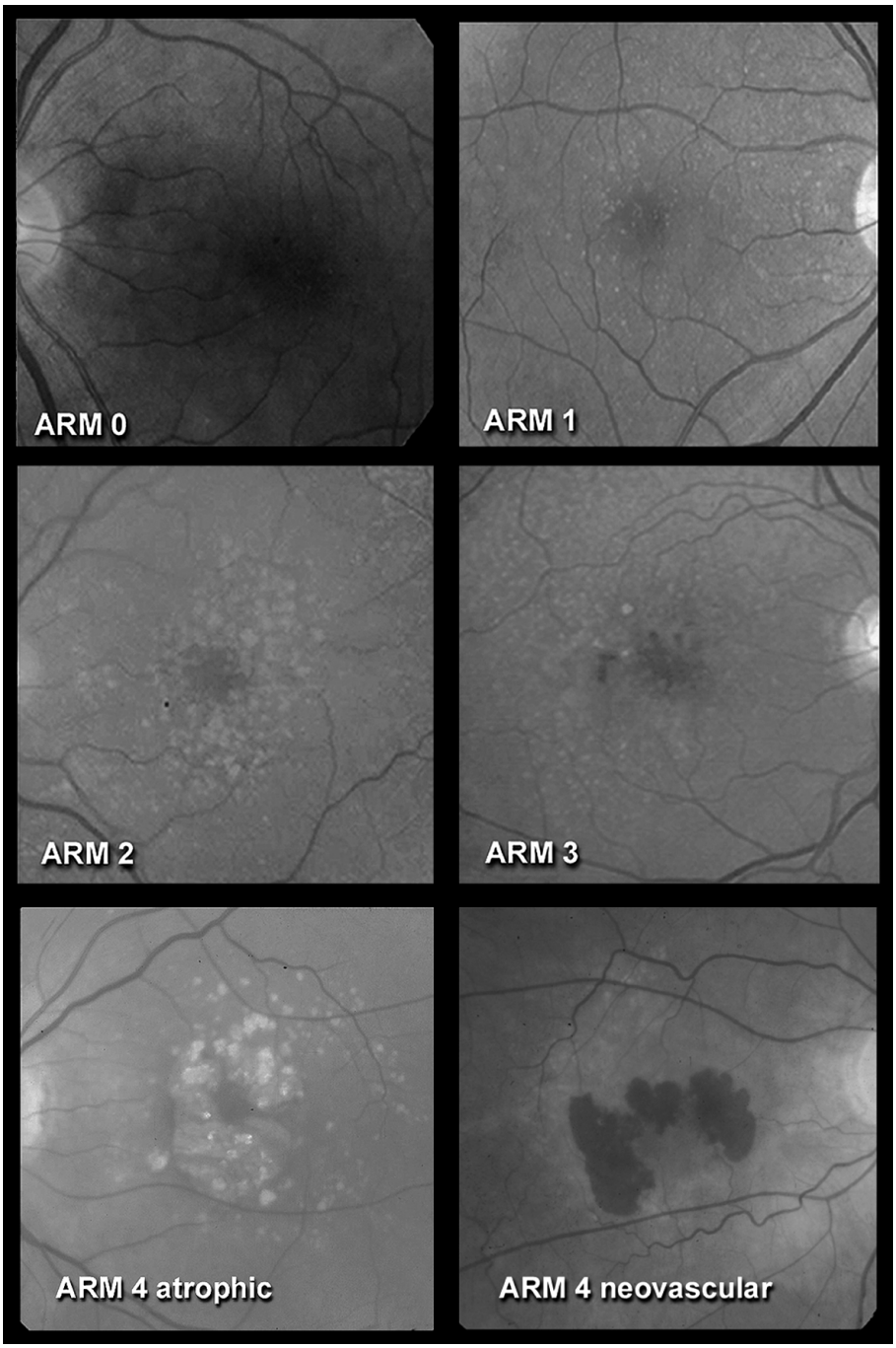


Figure 2.1.1 *Fundus photographs illustrating the mutually exclusive stages of age-related maculopathy (ARM) as used in the Rotterdam Study. Definitions of the ARM stages are given in Table 2.1.1. The same photographs in full colours are included in this thesis as an insert.*

Stages			Definitions
0	⎧	0a	No signs of ARM at all
	⎩	0b	Hard drusen (<63 μm) only
1	⎧	1a	Soft distinct drusen (≥63 μm) only
	⎩	1b	Pigmentary abnormalities only, no soft drusen (≥63 μm)
2	⎧	2a	Soft indistinct drusen (≥125 μm) or reticular drusen only
	⎩	2b	Soft distinct drusen (≥63 μm) with pigmentary abnormalities
3	-		Soft indistinct (≥125 μm) or reticular drusen with pigmentary abnormalities
4	-		Atrophic or neovascular age-related macular degeneration (AMD)

Table 2.1.1 *Classification of Mutually Exclusive Stages of Age-Related Maculopathy (ARM)*

subjects with unilateral AMD at baseline were included, using the unaffected eye.

Data analysis

The age-specific incidence rates of early ARM and AMD were obtained per five-year age-category by dividing the number of incident cases by the number of person-years within that age-category. The number of person-years was calculated by adding each person's contribution of follow-up time to the successive age-categories. So, one subject could contribute person-years to different age-categories. We assumed that early ARM or AMD started at the date of the first examination at which this diagnosis was made. Consequently, follow-up time ended on the date of screening. Confidence intervals (CIs) of incidence rates were calculated with Poisson SE. The cumulative incidence (actual risk per period) was derived from the incidence rate using the following exponential formula:

$$CInc(t) = 1 - e^{-IR * t}$$

where *CInc* is the cumulative incidence over a period of *t* years, *IR* is the incidence rate, and *e* is the constant 2.71828, the base of the natural logarithm.[160]

The predictive power of individual fundus signs for the incidence of AMD was calculated by a data set of one eye per subject, so that an affected eye was included from persons with incident AMD and a randomly selected right or left eye from all other subjects. Unaffected eyes of participants with unilateral AMD at baseline were also included. Subsequently, the incidences of

Baseline characteristics	First follow-up			Second follow-up		
	Died (n=359)	Refused, ungradable or lost to follow-up (n=1085)	Participated (n=4974)	Died (n=1308)	Refused, ungradable or lost to follow-up (n=1474)	Participated (n=3636)
Age-category (%)						
55-64 y	9.2	28.2	42.9	11.1	32.6	51.0
65-74 y	20.1	32.6	37.9	28.6	36.6	38.5
75-84 y	44.1	29.6	16.5	41.5	26.6	9.9
≥85 y	26.5	9.5	2.7	18.9	4.2	0.7
Gender (% female)	49.4	68.2	58.1	53.5	69.1	57.4
Institutionalised (%)	33.8	10.7	3.2	23.6	4.2	0.7
Hypertension (%)	46.9	40.9	33.1	46.6	38.9	29.5
Smoking (%)						
Current	27.9	24.5	22.5	26.3	24.5	21.4
Former	38.4	36.8	43.5	38.6	36.0	45.8
Stage of ARM* (%)						
Stage 1	30.7	28.9	27.3	29.3	29.1	26.6
Stage 2	8.9	7.4	5.7	10.4	6.4	4.5
Stage 3	3.1	2.3	0.9	2.8	1.7	0.6
Stage 4	9.2	1.5	1.1	4.9	1.8	0.4

*Definitions of the ARM stages are presented in Table 1.

Table 2.1.2 *Baseline characteristics of participants in the first and second follow-up examination.*

atrophic and neovascular AMD were calculated for each type of fundus sign or combination of signs.

An analysis of covariance adjusted for age and gender, when appropriate, was used to compare the baseline characteristics of participants and non-participants. All statistical analyses were performed with SPSS for Windows, Release 11.0.1.2001 (Chicago, SPSS Inc).

RESULTS

Incidence of ARM

The mean time between baseline and the first follow-up was 2.0 (median 2.0; SD 0.6) years, and between baseline and the second follow-up was 6½ (median 6.4; SD 0.4) years. Of 6418 participants with gradable photographs at baseline, 359 (5.6%) died before the first follow-up examination and another 949 (14.8%) died before the second follow-up examination. Of those alive at the first screening (n=6060), 52 (0.9%) were lost to follow-up, 987 (16.3%) refused to participate and 13 (0.2%) had ungradable photographs. Of those alive at the second follow-up (n=5110), 27 (0.5%) were lost to follow-up, 343 (6.7%) refused to participate and 51 (1.0%) had ungradable photographs. A comparison of general characteristics between participants and nonparticipants is provided in Table 2.1.2. Compared with participants, persons who were alive but were not included in the analyses were on average older, included more women, were more often resident in a nursing home, included more current smokers, and had a higher frequency of systemic hypertension ($P<.001$ for all). The difference in baseline prevalence of early ARM was considerable, but did not reach statistical significance ($P<.06$). During 26,592 person-years of follow-up, 47 subjects with incident AMD were identified, resulting in an overall incidence rate of 1.8 per 1000 person-years. The ratio of neovascular AMD-atrophic AMD was 1.4:1 (27 cases of pure neovascular AMD, 19 cases of pure atrophic AMD, 1 mixed case). The age-specific incidence rates of atrophic, neovascular and total AMD are shown in Table 2.1.3 and Figure 2.1.2. The rate of total AMD varied from 0 for those aged 55 to 59 years to 6.8 per 1000 person-years for those 80 years or older. The corresponding five-year risks were 0% and 3.4%, respectively. This increase over age was exponential when expressed on a logarithmic scale (data not shown). The incidence of neovascular AMD was somewhat higher than that of atrophic AMD, especially in the older groups.

In the cohort without early ARM at baseline, 413 subjects with newly developed early ARM at follow-up were identified. The incidence rate of early ARM increased with age, ranging from 1.4 per 1000 person-years (five-year risk, 0.7%) for those aged 55 to 59 years to 51.0 per 1000 person-years (five-year risk 22.5%) for those 80 years and older (Table 2.1.4).

Risk of AMD as a function of early fundus signs

From Table 2.1.5, the absolute five-year risk of AMD for an individual can be seen, stratified by stage of early ARM and age. For subjects with stage 0 ARM,

Age, y	Total Person-years	Atrophic AMD				Neovascular AMD				AMD, Total			
		No. Cases	Incidence Rate, per 1000 pyrs	95% CI	Five-Year Risk	No. Cases	Incidence Rate, per 1000 pyrs	95% CI	Five-Year Risk	No. Cases	Incidence Rate, per 1000 pyrs	95% CI	Five-Year Risk
55-59	2240	0	0.0	-	0	0	0.0	-	0	0	0.0	-	0
60-64	6218	0	0.0	-	0	1	0.2	0.0, 1.1	0.1	1	0.2	0.0, 1.1	0.1
65-69	6602	3	0.5	0.2, 1,4	0.2	2	0.3	0.1, 1.2	0.2	5	0.8	0.3, 1.8	0.4
70-74	5460	3	0.6	0.2, 1.7	0.3	7	1.3	0.6, 2.7	0.6	10	1.8	1.0, 3.4	0.9
75-79	3578	5	1.4	0.6, 3.4	0.7	9	2.5	1.3, 4.8	1.3	14	3.9	2.3, 6.6	1.9
≥80	2494	8	3.2	1.6, 6.4	1.6	9	3.6	1.9, 6.9	1.8	17	6.8	4.2, 11.0	3.4
Total	26592	19	0.7	0.5, 1.1	0.4	28	1.1	0.7, 1.5	0.5	47	1.8	1.3, 2.4	0.9

Table 2.1.3 *Age-specific incidence of atrophic, neovascular and total age-related macular degeneration (AMD).*

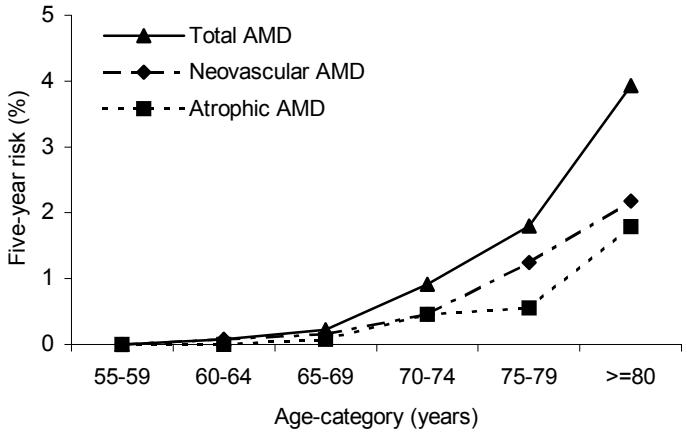


Figure 2.1.2 *Age-specific incidence in at least one eye of atrophic, neovascular and total age-related macular degeneration (AMD), expressed as five-year risk (percentage).*

the overall risk of AMD within a 5-year period was virtually absent, irrespective of age. For subjects with stage 1 ARM, the overall 5-year risk was 0.9%. However, this risk varied with age from 0.5% for those aged 60 to 69 years to 2.4% for those 80 years and older. Subjects with stage 2 ARM had an overall risk of 7.8%, which increased to 11.9% if they were 80 years or older. Finally, subjects with stage 3 ARM had an overall five-year risk of 28.0%, varying from 17.5% for those aged 60 to 69 years to 42.0% for those 80 years and older.

Risk of ARM as a function of sex

The crude incidence rate of AMD was 2.0 per 1000 person-years among men, and 1.6 per 1000 person-years among women. This difference did not reach statistical significance when corrected for age (rate ratio, 0.7; 95%CI, 0.4-1.2 [women versus men]), nor was there a significant difference in type of AMD. The sex-specific rates for early ARM were 17.1 per 1000 person-years for men, and 16.0 per 1000 person-years for women (rate ratio, 0.8; 95%CI, 0.7-1.0 [women versus men, adjusted for age]). In addition, the progression of ARM was not different for women versus men, when adjusted for age (rate ratio, 1.0; 95%CI, 0.9-1.2).

Natural course of ARM

The natural course of ARM is visualized in Figures 2.1.3, 2.1.4, and 2.1.5. Figure 2.1.3 projects for three age-categories the distribution of the five ARM stages at baseline and after 2 and 6½ years of follow-up. A slow but constant progression in ARM severity can be seen with advancing age and with time.

Age, y	Total Person-years	No. cases	Incidence rate, per 1000 pyrs	95% CI	Five-year risk
55-59	2179	3	1.4	0.4, 4.3	0.7
60-64	6085	32	5.3	3.7, 7.4	2.6
65-69	6376	69	10.8	8.6, 13,7	5.3
70-74	5102	97	19.0	15.6, 23.2	9.1
75-79	3212	102	31.8	26.2, 38.6	14.7
≥80	2159	110	51.0	42.3, 61.4	22.5
Total	25113	413	16.4	14.9, 18.1	7.9

*Early ARM is defined as the presence of either soft distinct drusen with pigmentary irregularities, or the presence of soft indistinct or reticular drusen (equal to ARM stage 2 plus 3).

Table 2.1.4 *Age-specific incidence of early age-related maculopathy (ARM)**

Likewise, the risk of each stage of ARM increased with age (Figure 2.1.4). This age-dependent increase in risk is similar for all stages, but the absolute risks differed between stages. In Figure 2.1.5, the change of each ARM stage at baseline separately is shown for the 2 follow-up examinations at 2 and 6½ years. It demonstrates that ARM evolved stage after stage with only few subjects skipping one stage during a short two-year period. The proportion of subjects who changed to a lower stage of ARM, and seemed to regress, was low, with a maximum of 8% (n=69) for the change from stage 1 to stage 0 within two years. At the second follow-up, 93% (n=64) of these subjects with presumed regression had returned to their baseline stage. The percentage of subjects who developed AMD during a 6½-year period was negligible for stages 0 and 1.

Next, we studied whether the advancement to atrophic and neovascular AMD resulted from differences in early fundus signs. Figure 2.1.6 shows the distribution of the ARM stages of subjects with incident atrophic or neovascular AMD at their examinations approximately 4½ and 6½ years before the onset of AMD. Atrophic AMD seemed to develop more often from stage 3 ARM (soft indistinct drusen with pigmentary abnormalities), while neovascular AMD also developed from stage 2 ARM (soft distinct drusen only). This difference, however, did not reach statistical significance (P=.25). The prognostic value of individual ARM fundus signs at baseline for the incidence of atrophic and neovascular AMD is given in Figure 2.1.7. The five-year risks of atrophic and neovascular AMD are plotted for each ARM fundus feature separately. Except for the percentage of grid area covered by drusen, no differences in baseline fundus signs were observed between the subtypes of AMD.

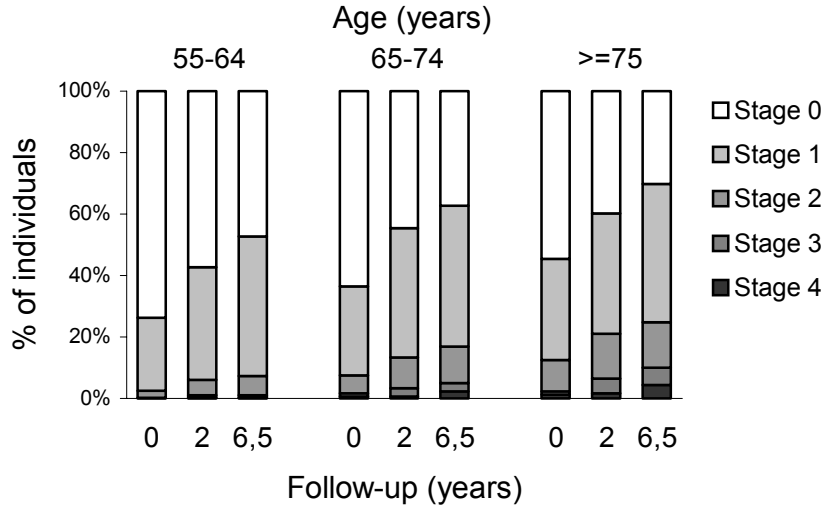


Figure 2.1.3 *Distribution of the 5 stages of age-related maculopathy (ARM) at baseline (0) and after 2.0 and 6½ years of follow-up, for 3 age categories separately. Definitions of the ARM stages are given in Table 1.*

Finally, to study the impact of pigment changes on the natural course of ARM, we plotted the five-year risk of AMD for subjects with or without the combination of drusen and pigment changes (Figure 2.1.8). The risk of AMD increased about three-fold when any of the three types of drusen (hard, soft distinct, or soft indistinct) was seen together with hyperpigmentation or hypopigmentation of the RPE.

Incidence in the second eye

Of 56 subjects who had prevalent uniocular AMD at baseline, 25 participated in the first follow-up examination and 7 participated in the first and the second follow-up examinations. In addition, 3 out of 9 subjects with incident uniocular AMD at the first follow-up examination participated in the second follow-up examination. In this sub-cohort of 35 persons with uniocular AMD (21 with neovascular AMD and 14 with atrophic AMD), 9 developed AMD in the second eye. This resulted in an incidence rate of 97.8 per 1000 person-years or a 5-year cumulative incidence of 38.7% (95%CI, 22.5%-60.9%). The type of AMD in the first eye was strongly related to the type of AMD in the second eye. All 5 subjects with uniocular neovascular AMD developed the same type of AMD in the other eye, as did 3 of the 4 subjects with uniocular atrophic AMD, resulting in 89% concordance between fellow eyes.

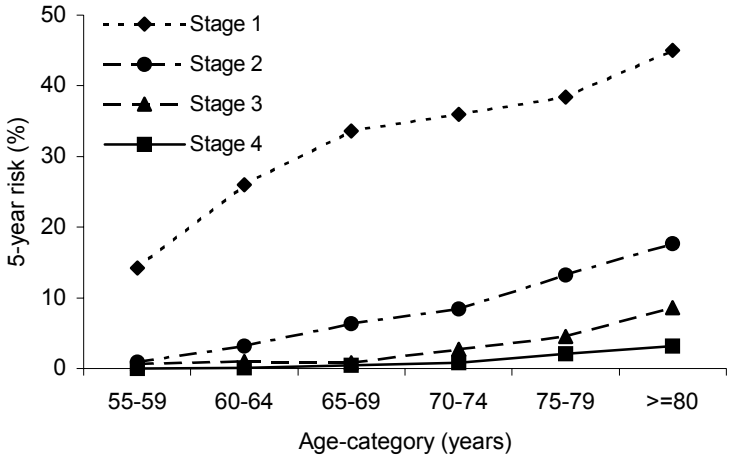


Figure 2.1.4 *Age-specific incidence in at least 1 eye of the stages of age-related maculopathy (ARM) as used in the Rotterdam Study, expressed as 5-year risk (percentage). Definitions of the ARM stages are given in Table 1.*

DISCUSSION

We studied the incidence and progression of ARM in a large, population-based cohort in the Netherlands during a 6½-year period. Our data demonstrate that the overall 5-year risk of atrophic or neovascular AMD in subjects 55 years and older is 0.9%, and the risk of early ARM is 7.9%. The incidence of AMD strongly depended on age and stage of ARM, reaching a maximum five-year risk of 42.0% for persons 80 years or older who were seen with soft indistinct drusen and pigment abnormalities. Sex was not an independent predictor of disease incidence or progression. The natural progression of ARM seemed to follow a distinct course, which we expressed as the succession of exclusive stages of disease with increasing risk of AMD. There were no significant differences in the early ARM fundus signs preceding atrophic or neovascular AMD.

Our study has merits and drawbacks. The design of the Rotterdam Study offered us the opportunity to investigate the development of ARM over a long period of time with many elderly people unaffected by the disease at baseline. The three successive examinations with short intervals enabled us to describe the step-wise progression of ARM. We minimized the potential for misclassification by the grading of fundus transparencies in a standard, well-established procedure by the same, well-trained graders. Moreover, the principal investigators of two other cohort studies that use the same grading method were approached to confirm the diagnosis of incident AMD.

	Age (years)			
	60-69	70-79	≥80	Total
Stage of ARM*				
0	0.0	0.1	0.0	0.0
1	0.5	1.1	2.4	0.9
2	3.0	9.2	11.9	7.8
3	17.5	22.5	42.0	28.0
Total	0.2	1.3	3.3	0.9

*Definitions of the ARM stages are presented in Table 1.

Table 2.1.5 *Five-year absolute risk (%) of age-related macular degeneration as a function of stage of early age-related maculopathy (ARM)* and age.*

Among the potential biases of a prospective cohort study is selective unavailability for follow-up. In our study, the percentage of subjects who were alive but refused to participate, had ungradable photographs, or were lost to follow-up in both follow-up examinations was 23.3% of the total cohort. This group was on average older, lived more often in a nursing home, and included more persons who smoked and had systemic hypertension. Moreover, non-participants had more severe stages of early ARM at baseline. This indicates that participants in the follow-up study were at a lower risk of developing AMD compared with the total eligible cohort, and that we have underestimated the incidence of AMD. Comparing the age-specific prevalence of ARM at baseline and at the second follow-up, we indeed found lower numbers for AMD in those older than 80 years, but not for early ARM. This effect could be explained by selective non-participation of subjects with clinically significant AMD (i.e., visual impairment). Considering the prevalence difference, the incidence in those 80 years and older may have been up to two times higher. There was no prevalence difference in those aged 70 to 79 years.

To provide meaningful risk estimates for clinical practice, and for comparisons of our data with those of others, we converted the incidence rates to five-year cumulative risks. For this conversion three assumptions need to be satisfied: a closed cohort, a small ratio of events per time to the population at risk, and no competing risk.[160] The first two criteria were fulfilled in our study, but the third criterion was not. Because only participants in the eye examination were included in the analyses, and participation is conditional on being alive, the considerable competing risk of death was not taken into account. For this reason, the actual five-year risk of AMD, which is most frequent at the oldest age, will be higher than that observed in our study. So, the five-year risks of AMD presented here are conditional on staying alive.

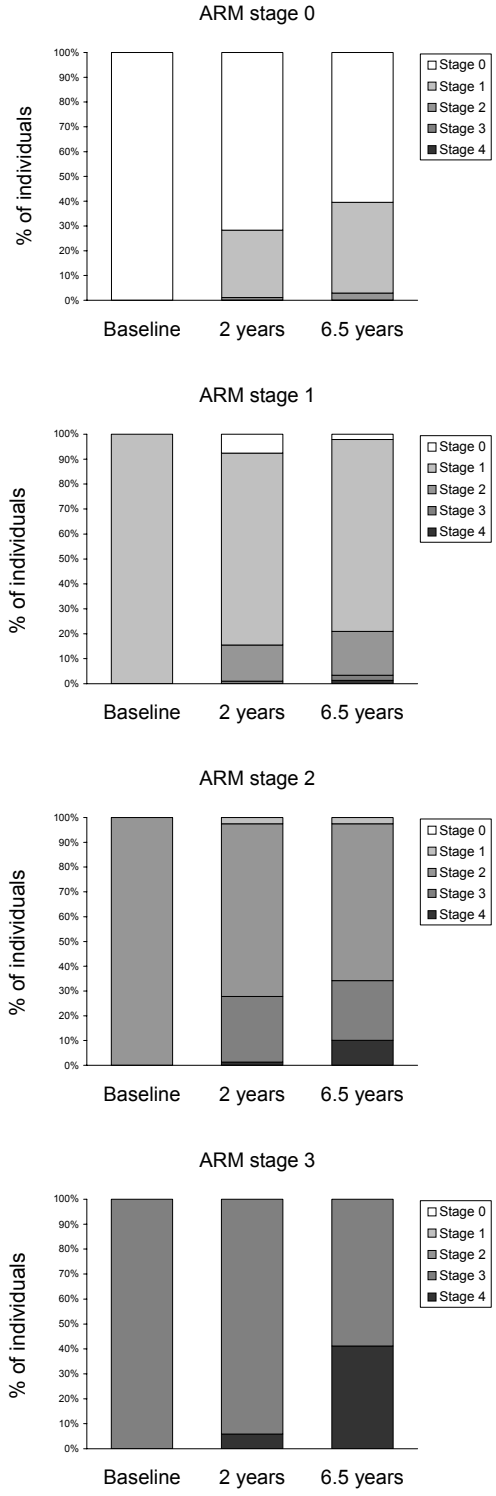


Figure 2.1.5 *Change over time of the stages of age-related maculopathy (ARM). In the first column, the total prevalence of each stage at baseline (0) is set at 100%. The second and third columns represent the relative proportions of the ARM stages as they emerged from this baseline stage at the first and second follow-up examination after a mean interval of 2.0 and 6½ years, respectively. Definitions of the ARM stages are given in Table 2.1.1.*

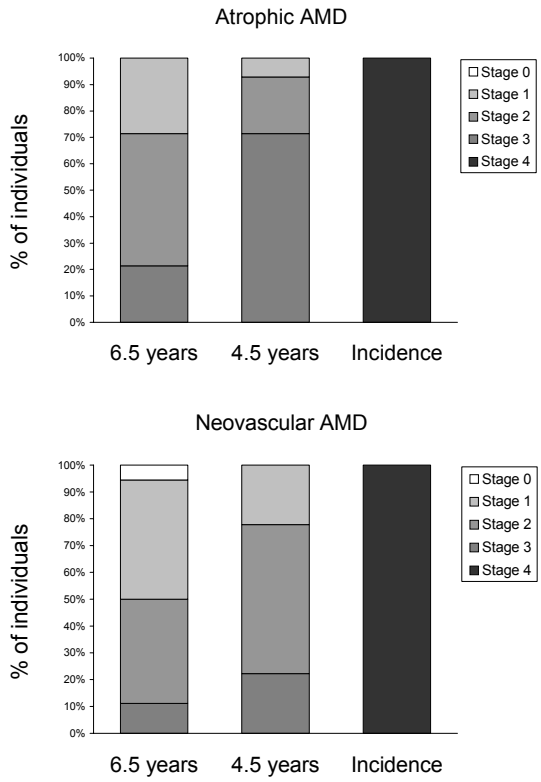


Figure 2.1.6 *Distribution of the stages of age-related maculopathy (ARM) prior to the incidence of atrophic and neovascular AMD. In the third column, the total number of incident cases at the second follow-up is set at 100%. The first and second column represent the relative proportions of the ARM stages as they were seen at the baseline and first follow-up examination with a mean interval of 6.5 and 4.5 years, respectively. Definitions of the ARM stages are presented in Table 2.1.1.*

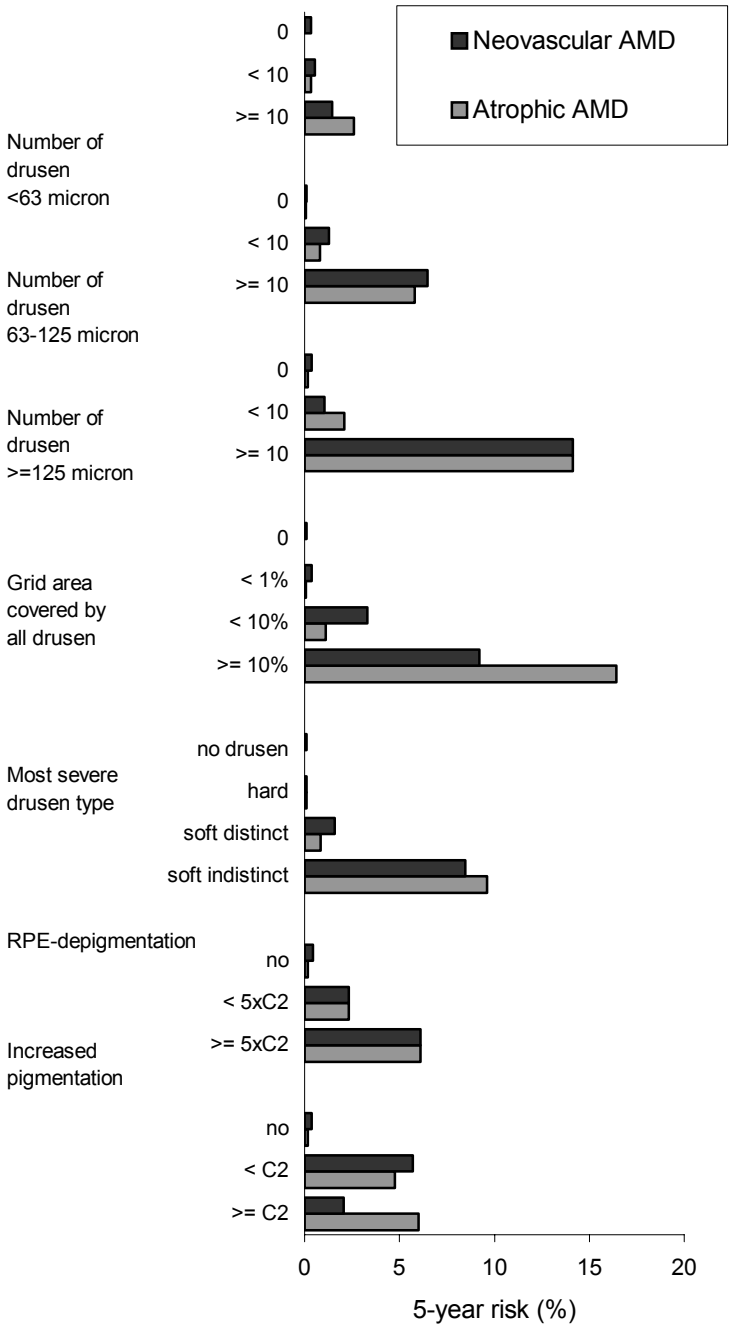


Figure 2.1.7 *Incidence of atrophic and neovascular age-related macular degeneration (AMD), expressed as five-year risks (percentage), for different categories of baseline early ARM fundus signs.*

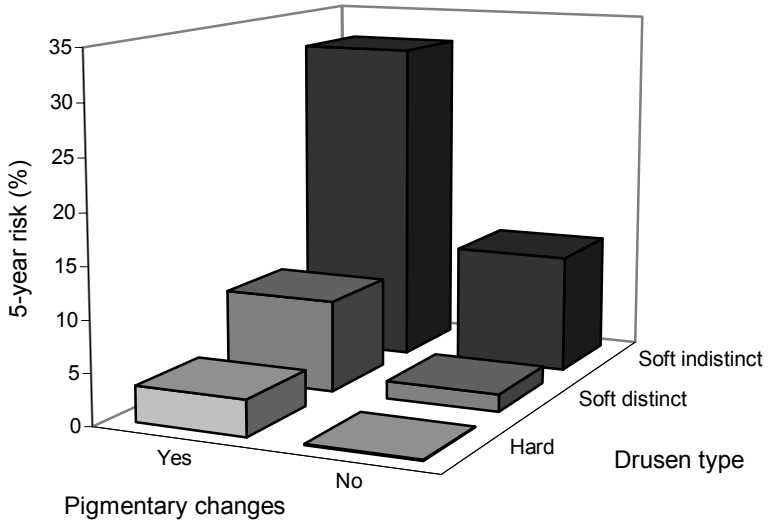


Figure 2.1.8 *Incidence of age-related macular degeneration (AMD) for the combination of baseline drusen type and pigment abnormalities, expressed as five-year risk (percentage).*

How do our data compare with those of others? In line with former findings, the incidence of AMD seems to be lower in the Rotterdam Study than in the Beaver Dam Eye Study.[116] The five-year incidence of late ARM for persons aged 65 to 74 was 1.3% in the US cohort, compared with 0.6% in our population. However, the design of the studies and the calculation of incidences are different and a comparison is therefore vulnerable to distortion. When we applied the same method and determined the age-specific incidence proportion in our cohort, the differences were less dramatic, but the incidence in the Beaver Dam cohort was still higher than in the Rotterdam cohort (for those aged 65 to 74 years, the incidence was 1.3% in Beaver Dam and 1.2% in Rotterdam; for those ≥75 years, the incidence was 5.4% in Beaver Dam and 2.4% in Rotterdam).[108, 209] Because the diagnostic procedures and definitions were similar, this difference seems to be real.

Comparing the progression rate from early ARM to AMD is also hampered by differences in ARM definition, time of follow-up, and age-composition of the cohort.[30, 32, 77] Recently, the results of the Age-Related Eye Disease Study (AREDS), evaluating the effect of supplementation with vitamins C and E, beta carotene and zinc on progression of ARM, were published.[8] The design of this multicentre clinical trial enabled an interesting comparison with our data, for subjects with clearly defined categories of ARM were enrolled and followed up for an average of 6.3 years. The AREDS AMD category 2, indicating intermediate drusen (≥63 to <125μm) with or without pigment abnormalities, is close to our stage 1, while the AREDS category 3,

indicating extensive intermediate or large drusen with or without pigment abnormalities may be considered similar to our ARM stages 2 and 3 combined. The probability of progression of AMD category 2 to advanced AMD was 2.0% after 7 years in the placebo group of the AREDS, while progression to AMD was 0.9% for those with stage 1 ARM in our study. The probability of advanced AMD in subjects with category 3 was 26.8% in the AREDS, while this risk for subjects with stage 2 or 3 ARM was 9.7% in the Rotterdam Study. The incidence of second eye involvement in subjects with uniocular AMD at baseline was 55.1% in the AREDS and 38.7% in the Rotterdam Study. These comparisons were not adjusted for age. Nevertheless, taking all considerations into account, comparison with the Beaver Dam Eye Study data and the AREDS findings seems to suggest that the progression and incidence rate of ARM are higher in a US compared with a European population. In an earlier report, it was shown that known risk factors such as smoking and cardiovascular disease could not explain the observed prevalence difference.[184] The elucidation of genetic and environmental factors that are accountable remains a challenge for the future.

We described the natural course of ARM by mutually exclusive stages with an increasing risk of AMD. This exercise confirmed previous thinking that drusen evolve from hard to soft distinct, and from soft distinct to soft indistinct. They increase in number and size, and confluence to form irregular plaques. Retinal pigment epithelial depigmentation and hyper-pigmentation appearing at this stage significantly enhance the risk of AMD. Deducting from our data, the type of AMD does not result from a significant difference in the course of early fundus signs, although extensive areas of drusen seem to have a slight predilection for the development of geographic atrophy. However, the number of incident cases of atrophic or neovascular AMD was low, and therefore the power to detect a statistically significant difference in baseline fundus signs may have been insufficient. The observed regression in ARM stage is most likely misclassification due to variability in photographic quality as a consequence of increasing media opacities. An argument supporting this explanation is the fact that most subjects with regression returned to the stage diagnosed at baseline at the next follow-up. However, a genuine temporary regression cannot be excluded.

In conclusion, the long-term follow-up of this large population-based cohort enabled us to estimate the absolute risk of AMD as a function of age and fundus signs. These data can be used effectively in the clinical care of patients, the design of clinical trials and other research objectives, and the establishment of risk profiles and strategies for future eye care. Most of all they give insight into the natural course of this disease.

2.2 Grading of age-related maculopathy for epidemiological studies: is digital imaging as good as 35-millimeter film?

Objective: *To evaluate the quality and reliability of grading age-related maculopathy (ARM) on stereo digital images as compared to stereo 35-mm colour film in the context of an epidemiological study.* ***Design:*** *Instrument validation study.* ***Participants:*** *Ninety-one subjects (137 eyes) with varying degrees of ARM, including no ARM.* ***Methods:*** *From both eyes of the participants, 35-mm film and digital stereoscopic fundus images were obtained with two identical Topcon fundus cameras. Two experienced graders classified all signs of ARM according to the International Classification System. Agreement between imaging techniques and between graders was calculated using the weighted κ statistic.* ***Main outcome measures:*** *Signs of ARM (number, size and morphologic characteristics of drusen; pigment changes; geographic atrophy; and neovascular macular degeneration), as well as an overall staging system of increasing ARM severity.* ***Results:*** *The weighted κ value for between-technique agreement ranged from 0.41 for number of drusen <63μm to 0.79 for drusen type and total area occupied by drusen. The κ values for atrophic and neovascular end-stage ARM were 0.87 and 0.94, respectively. The between-technique agreement on stages of ARM was approximately 0.76. The agreement between graders was largely the same for both imaging techniques.* ***Conclusions:*** *In the described setting, digital images were as good as 35-mm film for the grading of ARM. Considering the practical advantages of digital imaging, this technique may serve well in epidemiological studies of ARM.* ***Ophthalmology 2003;110:1540-1544.***

Research on age-related maculopathy (ARM) depends largely on a reliable method to detect the fundus abnormalities that characterize this blinding disease. In the early days of epidemiological studies of ARM, funduscopy was performed to ascertain the presence or absence of the disease. Later, fundus photography was introduced which permits detailed grading of transparencies. Stereoscopic color slides of the macular area are scrutinized in

order to describe and quantify drusen, pigment irregularities, and the end-stages of ARM, atrophic and neovascular age-related macular degeneration (AMD). Because studies have used varying terminology and a variety of grading methods, a standardized procedure of photography and grading was agreed on and adopted in 1995,[1] based on the Wisconsin ARM Grading System.[2] According to this international protocol, 30° or 35° stereoscopic colour transparencies centred on the fovea are taken after pupillary dilation.

With the advent of high-resolution digital imaging techniques and their incorporation into fundus cameras, there has been growing interest in the applicability of digital imaging to the study of ARM. There are a number of reasons to consider the use of digital imaging in epidemiological studies of ARM. Firstly, digital image quality is continuously improving and the current available resolution of these cameras may be adequate for the detection of drusen and other ARM-related fundus signs. Secondly, digital imaging permits the photographer to instantly judge the quality of the captured image and repeat the process if necessary. Thirdly, unlike 35-mm slides which occupy substantial storage space and have to be manually categorized for retrieval, digital images are stored conveniently on high capacity disks, are more easily transferred to the reading centre, and all the associated data can automatically be linked to the appropriate images. Finally, although digital cameras are presently more expensive, the costs per image are lower than for 35-mm film, and damage to the environment is minimized because of the avoidance of chemical processing, necessary for film-based photography.

These advantages were considered at the start of a new epidemiologic study on ARM, the EUREYE Study. This is a European Commission-funded population-based study of risk factors of ARM taking place in eight European countries. Before choosing a new technique fur fundus image capture, its reliability in comparison with the conventional method had to be proven. To the best of our knowledge, there have been no comparative studies that have formally compared digital and film images in ARM studies. We therefore wanted to compare ARM grading using 35-mm film with similar grading of digital images. We studied between-technique and between-grader agreement on the diagnosis of various fundus signs characteristic of early as well as late ARM.

METHODS

Subjects

Subjects included in this validation study were participants in the EUREYE study at two centres (Belfast and Rotterdam). Participants in this multi-centre study were recruited by random population sampling of people aged 65 and over and invited to attend a study clinic for an eye examination. Institutional review board approval was obtained at both the study centres and the study was conducted in accordance with the tenets of the declaration of Helsinki on research on human subjects.

For the current validation study fundus photographs were selected on the basis of their ARM status. Conditional on availability, an even distribution of ARM stages was selected that represented the whole range of ARM severity, including eyes with no ARM fundus signs. From 91 subjects, 35-mm slides and digital images of 137 eyes were selected. The quality of the slides and digital images varied, but none of them was ungradable.

Photography

Following pharmacological mydriasis (tropicamide 0.5% and phenylephrine 5%), both 35-mm film and digital 35° stereoscopic colour fundus images were obtained with a Topcon TRV-50VT fundus camera (Topcon Corporation, Japan). Images were taken by any of five photographers, two in the Belfast study centre and three in the Rotterdam study centre, all of whom have been trained in stereoscopic photography. The 35-mm colour transparencies were made using Kodak Ektachrome 64 ASA film (Kodak Ltd., England).

For digital images, the Sony 3CCD camera (Sony Electronics Inc., USA) with 800 x 600 pixel resolution for each of three colours and the Topcon ImageNet System 1.53 were used. The settings of the red and blue gain along with the gain adjustment minimum and maximum values are not standard and can affect the colour balance of the fundus photograph. To achieve optimisation of calibration we undertook a pilot study in which we assessed the colour gain and saturation of the images captured by the digital method in 30 subjects and compared them with images captured on 35-mm film. Optimal colour balance and contrast was found when the software was configured with red gain set to 05 and blue gain to 00. The minimum gain adjust was set to 136 and the maximum to 156. The software settings on all EUREYE study cameras were then configured in identical manner to ensure similarity of colour and contrast in the images captured in the different study centres. All images were stored as uncompressed TIF files.

Grading

Framed transparencies were mounted in plastic sheets and examined with a portable stereo viewer that provided 5x magnification on a tilted table viewing box with fluorescent back light (4000° K; Philips PL-W9 W/84, Philips Ltd., The Netherlands). Combined with a 2.5x magnification of the fundus camera the total magnification was approximately 12.5x. Digital images were examined on a Sony E500 21" FD Trinitron CRT monitor (0.24 mm aperture grille pitch). The monitor was set at 32 bits true colour and 1280x1024 pixel resolution at 103Hz. Stereo pairs were shown side by side on the monitor by the ‘compare images’ module of the ImageNet software. The digital stereo pairs were examined with a Topcon stereo viewer at about 50 cm distance. The monitor provided a 10x increase in image size, resulting in a total magnification, including that of the camera, of approximately 25x. No image manipulation was used before or during grading.

Stages of ARM			Definitions
0	⎧	0a	No signs of ARM at all
	⎩	0b	Hard drusen (<63µm) only
1	⎧	1a	Soft distinct drusen (≥63µm) only
	⎩	1b	Pigmentary irregularities only, no soft drusen (≥63µm)
2	⎧	2a	Soft indistinct drusen (≥125µm) or reticular drusen only
	⎩	2b	Soft distinct drusen (≥63µm) with pigmentary irregularities
3	-	3	Soft indistinct (≥125µm) or reticular drusen with pigmentary irregularities
4	-	4	Atrophic or neovascular age-related macular degeneration (AMD)

Table 2.2.1 *Definitions of mutually exclusive stages of age-related maculopathy (ARM)*

Two graders, each having eight years experience in ARM grading on stereo colour transparencies, were trained for two months in digital image grading. After this period, both graders randomly graded all 35-mm slides and digital images.

ARM definitions

Grading was done according to the definitions of the International Classification and Grading System for ARM.[1] In this system, all ARM signs within a fixed area (diameter 6000µm) around the fovea are recorded. The area is delineated by a grid, consisting of three concentric circles and a right angled cross at 45° and 135° to the horizontal, which is adapted to the magnification of the camera. The diameters of the central, inner and outer circle are 1000µm, 3000µm, and 6000µm, respectively. The fundus signs that were graded and included in this analysis were: number of drusen <63µm, ≥63-<125µm, and ≥125µm (0, <10, 10-19, ≥20); most severe drusen type (hard, soft distinct <125µm, soft distinct ≥125µm, soft indistinct, reticular); total area occupied by drusen (<1%, <10%, <25%, <50%, ≥50%, for central, inner and outer circle separately); confluence of drusen (no, <10%, <50%, ≥50%); hyper-pigmentation (no, <125µm, <175µm, ≥175µm) and hypo-pigmentation of the retinal pigment epithelium (no, <175µm, <5x175µm, <central circle, ≥central circle); atrophic AMD, and neovascular AMD. Atrophic AMD was defined as any sharply delineated area of apparent disappearance of the retinal pigment epithelium, larger than 175µm, with visible choroidal vessels, and in the

absence of neovascular AMD. Neovascular AMD was defined as the presence of a serous or hemorrhagic 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 vascular disease, such as diabetic retinopathy or chorioretinitis, or high myopia, trauma, congenital diseases, or photocoagulation for reasons other than for neovascular AMD, were excluded from ARM grading.

In order to check whether the grading of film or digital images made any difference on the overall outcome of ARM, we also looked at stages of ARM based on pooled fundus signs.[3] Definitions of these stages are given in Table 2.2.1. Two levels of detail were used, resulting in either five or eight stages of ARM. The first stage represents no ARM signs at all (stage 0a), the second stage represents only hard small drusen, the third to the seventh stage represent ARM with increasing levels of severity, and the last stage is similar to end-stage ARM or AMD.

Statistical analysis

For each eye, four scores were obtained by two different imaging techniques and two graders per image. No replicated imaging or grading was carried out.

The agreement in grading scores between imaging techniques and between graders was expressed in absolute percentages and calculated using the weighted κ statistic. For the interpretation of κ values, we followed the guidelines from Landis and Koch, slightly adapted by Altman[4]: <0.20 poor, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 good, and >0.81 very good agreement.

RESULTS

A comparison between both photographic techniques when grading individual ARM fundus signs is shown in Table 2.2.2, where the exact agreement, the agreement within one step, and the weighted kappa values are presented. For individual ARM fundus signs, the kappa value ranged from 0.41 for number of drusen <63µm to 0.79 for most severe drusen type and total area occupied by drusen. In general, lowest κ scores were found for the number of small drusen, and for pigment changes. The κ value for atrophic and neovascular AMD was 0.87 and 0.94, respectively. For the agreement on stages of ARM, the κ was 0.75 when eight categories were distinguished and 0.78 when five categories were distinguished. A comparison between digital images and 35-mm slides in the absolute scores for the eight stages of ARM for grader A and grader B separately is shown in the Figure.

The agreement between the two graders for each imaging technique separately is shown in Table 2.2.3. For 35-mm slides, the weighted κ for separate ARM fundus signs ranged from 0.45 for number of drusen <63µm to 0.80 for hyper-pigmentation. For digital images, the between-grader agreement ranged from 0.44 for drusen <63µm and hypo-pigmentation to 0.76 for hyper-pigmentation. With 35-mm film, the κ value for atrophic AMD was 0.80 and

	Number of Categories*	Agreement (%)	Agreement within 1 step (%)	Weighted κ
Number of drusen <63μm	4	59.7	92.3	0.41
Number of drusen 63-125μm	4	70.2	95.2	0.71
Number of drusen ≥125μm	4	78.5	95.0	0.68
Most severe drusen type	5	75.3	94.7	0.79
Total area occupied by drusen	5	86.2	97.8	0.79
Confluence of drusen	4	75.4	87.7	0.70
Hyperpigmentation	4	74.1	82.7	0.60
Hypopigmentation	5	79.8	91.2	0.61
Atrophic AMD	2	97.8	100	0.87
Neovascular AMD	2	98.9	100	0.94
Stage of ARM†	8	62.8	81.1	0.75
	5	71.9	96.8	0.78

ARM = age-related maculopathy; AMD = age-related macular degeneration
*Slightly modified from the International Classification and Grading System[1]
†For definitions see Table 2.2.1

Table 2.2.2 *Comparison of digital images with 35-mm film for grading various fundus signs of age-related maculopathy. Results from both graders are combined.*

for neovascular AMD 0.88, compared to 0.76 and 0.83, respectively, when grading the digital images. The κ value for stages of ARM was 0.78 for film and 0.72 for digital images.

DISCUSSION

We studied the reliability of grading ARM using stereo digital images, as compared to conventional grading of colour 35-mm slides. Our study showed that there were no important differences between both photographic techniques in the grading results. The between-technique agreement ranged from good to very good, and the between-grader agreement was about the same for both techniques.

The grading results we presented were based on the unadjusted results from each grader separately. We did not correct the results after discussion among graders and supervisors of questionable results. Such an adjudication procedure of all questionable results and all potential AMD cases is normally always performed. Therefore, the unadjusted results represent an underestimation of the final level of agreement that is commonly reached.

For the grading of 35-mm slides, which is current practice, our between-grader agreement is comparable with that of other studies. Klein et al. have

Grader A, Weighted κ 0.74

		Digital Images								
	ARM stage	0a	0b	1a	1b	2a	2b	3	4	Total
35-mm Film	0a	3	2	3			1			9
	0b	7	11	4						22
	1a	1	2	27	3	5	3	1		42
	1b				3					3
	2a			2		14	1	3		20
	2b			1	3	1	6	1		12
	3			1		1	2	4	1	9
	4					1			19	20
	Total	11	15	38	9	22	13	9	20	137

Grader B, weighted κ 0.75

		Digital Images								
	ARM stage	0a	0b	1a	1b	2a	2b	3	4	Total
35-mm Film	0a	6	5	2						13
	0b		11	6	1		2			20
	1a		8	26		4				38
	1b		1	1	1		2			5
	2a			3		6	3	3		15
	2b		1	2	1	2	10			16
	3			1		2	1	5		9
	4						1		20	21
	Total	6	26	41	3	14	19	8	20	137

Figure 2.2 *Comparison of grading eight stages of age-related maculopathy on digital images or 35-mm slides by grader A and grader B. Agreement is expressed in absolute numbers.*

published the agreement between graders on specific ARM lesions based on the Wisconsin ARM Grading System.[2] Their weighted κ value for drusen size was 0.68, for drusen type 0.65, and for drusen area 0.71. Their kappa values for subretinal fibrous scar and geographic atrophy were 0.48 and 0.87, respectively. Our results on these same ARM signs were 0.74 for drusen size, 0.78 for drusen type, 0.70 for drusen area, 0.88 for neovascular AMD, and 0.80 for atrophic AMD. It should be noted, however, that the Wisconsin system distinguished more categories per ARM sign than we did (6-7 compared to 4-5). Other studies that have reported interobserver agreement in ARM grading found weighted kappa values in the same range, from 0.48 to 1.00,[5] or from 0.64 to 0.93.[6]

	Number of categories*	35-mm film		Digital images	
		Agreement (%)	Weighted κ	Agreement (%)	Weighted κ
Number of drusen <63µm	4	60.9	0.45	64.4	0.44
Number of drusen 63-125µm	4	72.1	0.72	66.9	0.67
Number of drusen ≥125µm	4	70.0	0.63	75.8	0.70
Most severe drusen type	5	72.6	0.78	70.7	0.67
Total area occupied by drusen	5	76.8	0.70	74.7	0.68
Confluence of drusen	4	72.6	0.60	76.0	0.73
Hyper-pigmentation	4	86.1	0.80	82.3	0.76
Hypo-pigmentation	5	81.1	0.58	75.0	0.44
Atrophic AMD	2	97.1	0.80	96.3	0.76
Neovascular AMD	2	97.9	0.88	97.1	0.83
Stage of ARM*	8	65.7	0.78	59.0	0.72
	5	72.3	0.79	64.9	0.74

ARM = age-related maculopathy; AMD = age-related macular degeneration
*Slightly modified from the International Classification and Grading System[1]
*For definitions see Table 2.2.1

Table 2.2.3 *Comparison of grader A with grader B for grading various fundus signs of age-related maculopathy on 35-mm film or digital images.*

To the best of our knowledge, this is the first study to evaluate the potential of digital photography for the grading of ARM. Different groups have examined digital imaging techniques in the screening of diabetic retinopathy.[7-12] Most of these studies suggested that digital photography is equivalent or superior to film based image capture[7-9,12] or contact lens biomicroscopy.[11] One study using a nonmydriatic camera concluded that digital fundus imaging is inferior to 35-mm slide imaging.[10] It should be noted that the early signs of ARM, which include patchy atrophy and small drusen, are more difficult to detect and grade than the fundus signs of diabetic retinopathy.

In the present study, image capture was achieved using the Sony 3 chip colour camera. This provides a resolution equivalent to 800 x 600 pixels. At the time of inception of EUREYE this camera was selected as it represented best value for money and the captured images were of equivalent or better quality to other existing fundus cameras. However, further improvements in digital technology have resulted in cameras with higher resolution, which we believe will make photograding of ARM changes even more reliable.

Both graders expressed a strongly positive preference for the grading of digital images. Their posture when viewing the computer monitor was far better than the position when viewing slides with a stereo magnifier on an oblique table viewer box. Also, retrieval of digital data was preferred over the traditional procedures with slides. Preparation time, which is considerable for framed slides, was negligible for digital images, whereas grading time was about the same.

Some aspects need consideration in order to maintain the quality of grading digital images. Obviously, photographers and graders should be trained extensively, and settings of camera, software and monitor should not be changed during the study. Still, because minor alterations may develop in colour projections, regular calibration of the camera and monitoring system is necessary. The same holds more or less for 35-mm film, where manufacturing and developing procedures may change over time, although probably on a larger time scale than changes in a video camera and monitor. Careful storage of digital information with regular backups is necessary to avoid losing images.

In some eyes with lightly pigmented choroids we found variation in the fundus pigmentation in subsequent digital images of the same eye. In these cases the background pigmentation seemed to vary between images not because of differences in flash intensity but because of alignment of the camera. Although this does not seem to interfere with the overall ARM grading, it may have some influence on the grading of pigment changes as well as that of small drusen. This may be the explanation for the lower κ scores for between-technique agreement on the number of drusen $<63\mu m$ and on pigment changes. However, we think that this phenomenon also plays a role in film-based photography, as can be concluded from the relatively low kappa scores on these same items.

In this study the reliability of digital imaging for the purpose of grading ARM, at least in our setting, was established. Considering the practical

advantages and low costs per image, this new technique might prove valuable in clinical and epidemiological studies. We would like to emphasize, however, that our conclusions are only pertinent to colour fundus photography for the international ARM grading system in epidemiological studies. The validity of using digital colour photography and angiography in the clinic or in clinical trials is beyond the scope of this study.

3
Risk factors

3.1 The relationship between refraction and prevalent as well as incident age-related maculopathy

Purpose: *To study the relationship between baseline spherical equivalents (SphE) of refraction and prevalent as well as incident age-related maculopathy (pARM and iARM, respectively).* ***Methods:*** *The study was performed as part of the Rotterdam Study, a population-based, prospective cohort study. The SphE (in dioptres), measured with autorefraction and subjective optimisation, was recorded in 6209 subjects aged 55 years or older. Aphakic or pseudo-phakic 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 pigment changes, or indistinct drusen, or atrophic or neovascular age-related macular degeneration (AMD). For the prevalence analyses, ARM was classified into no, p(early)ARM, or pAMD, and in each subject the eye with the most advanced ARM and the corresponding refraction was selected. After a mean 5.2 years of follow-up, 4935 subjects had complete data for the incidence analyses. In each subject, the eye with iARM was selected.* ***Results:*** *The age- and gender-adjusted odds ratio (OR) of pARM (n = 536) for every dioptre towards hyperopia was 1.09 (95% conficence interval [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). For each dioptre 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.* ***Invest Ophthalmol Vis Sci 2003, in press***

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 pigment changes at the level of the retinal pigment epithelium (RPE).[148] Its end-stage, also called age-related macular degeneration (AMD), is the most important cause of incurable visual impairment in the Western world. It has been proposed that these drusen

are a manifestation of dysfunction and degeneration of the RPE and retina.[148] 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 AMD.[2, 31, 146] In a small number of subjects with neovascular AMD, laser photocoagulation and photodynamic therapy can be successfully used 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.[9] In view of preventive measures, better knowledge of pathophysiology and detection of early stages is important.[200]

Different studies have looked at various risk factors involved in AMD, such as smoking, atherosclerosis, and genetic factors.[54] Similarly, it has been hypothesized that ocular factors, such as cataract (extraction), iris colour, and refractive errors may be involved in the development of this disease.[118, 198] An association between hyperopia and AMD was first described by Maltzman et al. in 1979 in a case-control setting.[133] After that, a few other case-control studies on this topic showed conflicting results.[3, 7, 83, 163] One report of a population-based cross-sectional study mentioned a weak association between hyperopia and early ARM.[214] More recently, a population-based follow-up study showed no relationship between refractive error and 10-year incidence of early ARM and AMD.[228]

Becauses the nature of the association between refraction and ARM is still unclear and may 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.[74] 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[208]. Participants underwent a full eye examination, including stereo 35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) centred 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[24]. 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 hyper-pigmentation, and 0.58 for hypo-pigmentation.

ARM was defined as the presence of either large (≥63 μm) soft distinct drusen with pigment 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 fovea 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. Ophthalmologists trained the medical doctors performing these measurements 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 or physiological miosis. The value of their spectacles (if present) was taken as measured with a lens meter (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 dioptres.

	Advanced myopia*	Myopia†	Emmetropia‡	Hyperopia§	Advanced hyperopia‖
Number	443	770	966	3180	850
Mean 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 (dioptre)	- 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

Table 3.1.1 *Baseline characteristics and prevalence of early ARM and AMD per stratum of refraction, expressed in spherical equivalents (SphE).*

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 pigment 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

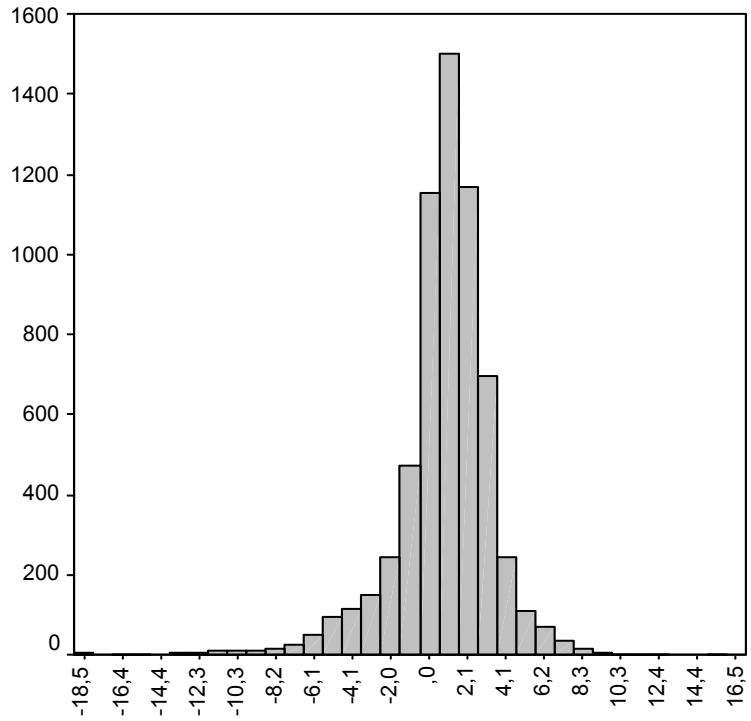


Figure 3.1 *Distribution of refraction at baseline (n = 6209).*

participants at baseline on whom we had complete data were included in the follow-up analyses, resulting in 497 cases with iARM. Incidence of ARM was defined as absence of 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 6½ years. The overall mean follow-up time was 5.2 years.

Statistical Analysis

Analyses were started by taking the SphE in dioptres 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 analysed 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.

	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 dioptre towards hyperopia
‡See legends Table 3.1.1

Table 3.1.2 *Odds ratios (OR) of prevalent ARM according to spherical equivalents of refraction*.*

	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 3.1.1
† Due to the low number of iAMD cases, they are not presented separately

Table 3.1.3 *Incidence of ARM per stratum of refraction in absolute numbers. (n = 4935)*

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 modelling was performed to establish the relationship of baseline 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 Figure 3.1 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 dioptre 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 dioptre 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% 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 dioptre 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 dioptre towards hyperopia increased the risk of iARM with 5% (OR, 1.05; 95%

	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 dioptre towards hyperopia
‡See legends Table 3.1.1

Table 3.1.4 *Odds ratios (OR) of early incident ARM according to spherical equivalents of refraction*.*

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 dioptre 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 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.[198] To avoid misclassification of refractive status, these subjects had to be excluded. The excluded subjects at baseline were on average older and more often institutionalised. 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 nonparticipation, it is in our opinion unlikely that this nonparticipation 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 empiric 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 cutoff SphEs were chosen after considering the distribution of refraction in this population. When the analyses were repeated using other cutoffs, we saw similar results (data not shown). We decided to take the presented cutoff 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 analysed our p(early)ARM and pAMD cases to compare our results with those in other studies. One report from a population-based cross-sectional study mentioned a weak association between hyperopia and only early ARM[214] (per dioptre towards hyperopia OR, 1.1, 95% CI, 1.0-1.2). The case-control AREDS study[7] showed an association between hyperopia and large drusen as well as with neovascular and atrophic AMD. However, recently a population-based follow-up study[228] showed no relationship between refractive status and the 10-year incidence of ARM. 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 may 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 must be considered. Drusen in myopic fundi may be more difficult to assess because the usually fairer RPE and choroidal pigmentation results 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 status of that eye. A photograph 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 may 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 we found between hyperopia and neovascular pAMD (per dioptre 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.

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 and more rigid and compact sclerae.[26] This generalized stiffness of the sclera may cause an increase in resistance of the choroidal venous outflow[61, 59] with throttling of the

vorticose veins, and a thicker choroids.[92, 157] Both histologic and in vivo studies with laser Doppler flow measurements have shown an increased choroidal resistance in AMD-cases compared with gender- and age-matched controls.[23] 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[40] in the pathogenesis of ARM remains unclear.

Other speculations that may explain the observed association with hyperopia may be that a poorer cooling of the retina by an impaired choroidal blood flow may lead to a higher susceptibility to 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 square millimeter of the fovea or per RPE cell is different in hyperopia.

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[54], the risk of 5-9% per dioptre towards hyperopia is still a considerable increase. In view of these findings, special attention may have to be given to older persons with hyperopia (e.g., over 70 years with hyperopia >3 dioptre) in order to offer them in the presence of ARM a 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.

3.2 Blood pressure, atherosclerosis, and the incidence of age-related maculopathy

Purpose: *To determine whether blood pressure and subclinical 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 aged 55 years or more, were free of ARM, and 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 pigment changes, or indistinct drusen, or atrophic or neovascular age-related macular degeneration.* ***Results:*** *After a mean follow-up of 5.2 years, incident ARM was diagnosed in 417 subjects. 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 mm Hg 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 1 SD, 1.15; 95% CI 1.03-1.28) increased the risk of ARM. The lowest compared with the 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.* ***Conclusion:*** *Elevated systolic blood or pulse pressure or the presence of atherosclerosis may increase the risk of development of ARM.* ***Invest Ophthalmol Vis Sci 2003, in press***

In a recent report, the National Eye Institute estimated that currently 1.6 million Americans have age-related macular degeneration (AMD), the most common cause of incurable blindness and visual impairment in industrialized countries.[58, 114] Because of the 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.[2, 31] Prevention of AMD is hampered by a lack of knowledge about aetiology and modifiable risk factors.[54] Only high-

dose supplementation with specific antioxidant nutrients has been shown to slow the development of AMD.[8]

Already in 1937, Verhoeff and Grossman postulated that systemic vascular factors may be involved in the pathogenesis of AMD.[161] More recently, interest in this potential relationship has grown,[186] and a vascular model has been proposed in which a process that resembles atherosclerosis causes an accumulation of lipids and subsequently an increase in choroidal vascular resistance.[62] This process would interfere with the high metabolic rate of the retinal pigment epithelium and lead to the development of sub-retinal deposits (drusen), pigment abnormalities, and, finally, 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.[24]

Most epidemiologic studies have addressed the vascular hypothesis by studying the classic risk factors for cardiovascular disease, such as blood pressure, serum cholesterol, and smoking, as well as clinical manifestations of atherosclerosis, such as myocardial infarction. Except for smoking, the results have been inconclusive.[3, 7, 50, 73, 82, 111, 115, 181, 189, 207] 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, used direct measurements of atherosclerosis.[120, 209]

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

METHODS

Population

Information on the identification and description of the baseline study population has appeared in previous reports.[209] Briefly, the Rotterdam Study is a population-based prospective cohort study of the frequency and determinants of common cardiovascular, locomotor, neurological, and ophthalmologic diseases.[74] 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 Centre 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.[209] Participants underwent a full eye examination, including stereo 35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) centred 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.[24] 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 hyper-pigmentation, and 0.58 for hypo-pigmentation.

ARM was defined as the presence of either large (≥63 μm) soft distinct drusen with pigment 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 neuroretinal or RPE detachment and/or a subretinal neovascular membrane and/or a subretinal haemorrhage, 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 diagnosis.

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 centre, height and weight were determined and non-fasting blood samples were drawn. Serum total cholesterol and high-density lipoprotein (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

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

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 atherosclerosis.

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.[27] 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 digitised 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 thickening 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.[225] 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 with both the continuous (ankle-arm index and carotid wall thickness) and categorical measurements (number of carotid plaques and aortic calcifications). To do so, we transformed all variables to a 10-point scale. For the continuous variables, deciles were created, and subjects received 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 two locations (57% of the population had less than two locations), and 1 cm to less than 2.5 cm of aortic calcifications (43% had less than this), had 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 pigment 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

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

to participate, and 13 had ungradeable 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 ungradeable 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 analysed 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 analysed both continuously (in number of plaques) and in categories. Aortic calcifications were studied in categories only and the atherosclerosis composite score was analysed 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).

		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

Table 3.2.3 *Adjusted odds ratios of age-related maculopathy associated with ankle-arm index.*

RESULTS

The baseline characteristics of the eligible study cohort, adjusted for age and gender, are presented in Table 3.2.1. 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 at baseline.

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 pigment irregularities (n=261), or indistinct drusen without (n=109), or with pigment 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 pigment 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 3.2.2, the odds ratios (OR) of incident ARM associated with 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 3.2.3 presents the association between ankle-arm index and risk of ARM. Peripheral atherosclerosis was not associated with ARM. However, when analysing 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.

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

Table 3.2.4 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 3.2.5. 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 3.2.6, the analysis of the composite score of atherosclerosis is presented. The highest score compared with the lowest carried an OR 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. Because subjects with early stages at baseline were excluded, AMD developed in very few (n=14) 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 investigators.[5] A separate analysis of those cases with only neovascular AMD as outcome was not possible because of the small sample. 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.[116, 200]

		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

Table 3.2.5 *Adjusted odds ratios of age-related maculopathy associated with aortic calcifications.*

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 epidemiologic studies relies on fundus photography,[24] 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 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. Because these reasons were not related to carotid wall thickness, we do not think that this biased our results. Still, it is possible that some error 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 relationship between ankle-arm index and atherosclerosis seems well established.[55] Increase in the carotid intima-media thickness may also reflect hypertrophy of the vessel wall as a response to hypertensive stress. Because many studies have shown that increased carotid wall thickness is associated independently of hypertension with both cardiovascular risk factors and cardiovascular events, it can be regarded as a valid indicator of atherosclerosis.[27, 162]

An association between blood pressure or hypertension and prevalent AMD was reported earlier in three case-control studies: the Eye Disease Case-Control Study,[3] the AMD Risk Factors Study,[82] and the Age-Related Eye Disease Study.[7] Also, the Framingham Eye Study found an association between prevalent ARM and hypertension diagnosed 25 years before.[189] On the contrary, no association between blood pressure and prevalence of ARM was found in several population-based studies.[50, 73, 120, 111, 181, 207] The only prospective, population-based study of this association so far, the Beaver Dam Eye Study, found that both systolic blood pressure and hypertension were significantly related to the incidence of RPE depigmentation, but not of drusen.[115] Loss of statistical power may 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 Atherosclerosis Risk in Communities study, in which data on carotid intima-media wall thickness and carotid plaques were available.[120] They found a statistically significant association between carotid plaques and retinal

		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)
	4rd	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

Table 3.2.6 *Adjusted odds ratios of age-related maculopathy associated with a composite score of atherosclerosis.*

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.[110] 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.[209] 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, whereas 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 in the ophthalmic artery and in the choroidal and retinal circulation. Considering the low prevalence of carotid stenosis in our cohort, this explanation seems implausible. 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 results 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 RPE, 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.[62] A decreased choriocapillary density was demonstrated in ageing human eyes, especially in those with ARM.[157] Moreover, in patients with ARM, reduced choroidal perfusion was shown by direct measurement of the choroidal blood flow.[60, 65, 69, 154] In summary, multiple lines of evidence suggest a role for atherosclerosis in the pathophysiology of ARM.

The question should be answered of 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 may be that high blood pressure and atherosclerosis, independent of each other, increase the risk of ARM. However, because 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 defence could disturb the association between atherosclerosis and ARM. Because oxidative stress is implicated both in the aetiology of ARM[19] and in the pathogenesis of atherosclerosis,[86] 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 sub-clinical 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.

3.3 Cholesterol and age-related maculopathy: is there a link?

Purpose: *To examine the relation between serum cholesterol, apolipoprotein E genotype (APOE), and the risk of age-related maculopathy (ARM).* ***Design:*** *The Rotterdam Study, a population based prospective cohort study.* ***Methods:*** *Serum levels of total and high-density lipoprotein (HDL) cholesterol as well as APOE genotype were determined at baseline. In a total of 3944 subjects, 400 subjects were diagnosed with incident ARM after a mean follow-up of 5.2 years.* ***Results:*** *Serum HDL but not total cholesterol was associated with an increased risk of ARM (OR per SD 1.20; 95% CI 1.06-1.35). The association remained unchanged after adjustment for APOE genotype. When stratifying for APOE genotype, the association was strongest in persons with the ε4 allele, while an inverse association seemed to be present for ε2 carriers.* ***Conclusion:*** *Elevated HDL but not total cholesterol is associated with an increased risk of ARM. APOE genotype does not explain this association, but may be an effect modifier.* ***Submitted for publication***

Drusen and atherosclerosis are determinants of age-related maculopathy (ARM).[204] Cholesterol is an important constituent of drusen and is involved in the pathogenesis of atherosclerosis.[148] Although a link between ARM and serum cholesterol seems plausible, epidemiological studies failed to demonstrate a relation. Many studies did find elevated levels of high-density lipoprotein (HDL) cholesterol in patients with ARM, but no explanation has been provided so far.[82, 111] Since the cholesterol transporter apolipoprotein E gene (APOE) is associated with ARM[94] and influences cholesterol levels, our aim was to examine the association between serum cholesterol and incident ARM, and to evaluate whether this association is influenced by APOE genotype.

This study was performed in the Rotterdam Study, a population-based prospective cohort study of persons aged 55 years and older.[200] Incident ARM was defined as the development of soft distinct drusen with pigmentary irregularities, indistinct drusen, or atrophic or neovascular ARM. Serum levels of total and HDL, but not LDL, cholesterol and APOE genotype were determined at baseline. Categories of cholesterol were defined using 1 standard deviation (SD) from the mean as cut-off value. APOE genotypes with the ε2 or

	mg/dL	Total	Cases	Odds Ratio (95% Confidence Interval)		
				Model 1*	Model 2†	Model 3‡
Total cholesterol						
Per SD	46.3	4776	414	0.95 (0.85-1.05)	0.96 (0.85-1.08)	0.96 (0.84-1.09)
Per category§	<212.4	764	77	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	212.4-305.0	3285	284	0.95 (0.72-1.24)	1.10 (0.79-1.54)	1.11 (0.79-1.56)
	>305.0	727	53	0.80 (0.55-1.17)	0.80 (0.51-1.27)	0.80 (0.50-1.29)
High-density lipoprotein cholesterol						
Per SD	15.4	4766	414	1.19 (1.05-1.33)	1.19 (1.03-1.38)	1.16 (1.00-1.35)
Per category§	<38.6	982	74	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	38.6-65.6	2913	256	1.31 (0.99-1.73)	1.38 (0.99-1.91)	1.29 (0.92-1.79)
	>65.6	871	84	1.53 (1.08-2.16)	1.69 (1.11-2.58)	1.55 (1.01-2.39)

*Adjusted for age, gender, and time of follow-up
†Additional adjustment body mass index, smoking, systolic blood pressure, atherosclerosis, and alcohol intake
‡Additional adjustment for APOE genotype
§Defined using 1 standard deviation from the mean as cut-off value

Table 3.3 *Association between baseline serum levels of total and high-density lipoprotein cholesterol and incident age-related maculopathy.*

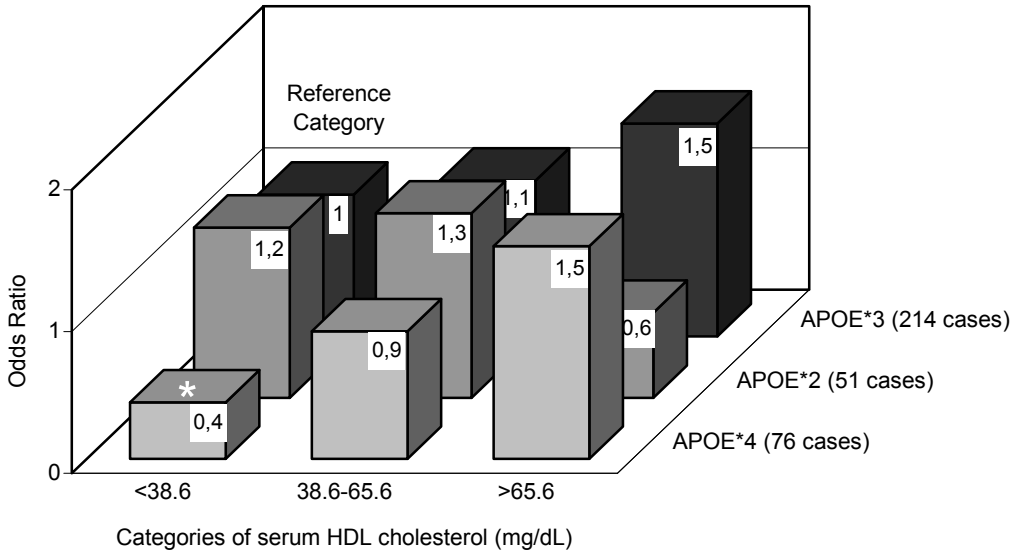


Figure 3.3. *Interaction between serum cholesterol level and apolipoprotein E genotype (APOE) in the association with incident age-related maculopathy. APOE genotypes with the ε2 or ε4 allele were grouped. The reference category was defined as the E3E3 genotype with the low cholesterol category. The asterisk marks a statistically significant association at the P<.05 level.*

ε4 allele were grouped, while the E3E3 genotype was the reference. We performed logistic regression analysis with adjustment for age, gender, time of follow-up, body mass index, smoking, systolic and diastolic blood pressure, atherosclerosis (composite score of 4 non-invasive measurements), and alcohol intake.

Of the 5836 persons at risk of ARM, 4822 (83%) participated at follow-up. Complete data were available on 3944 subjects (68%). After 5.2 (SD 2.1) years of follow-up, 400 subjects were diagnosed with incident ARM. No significant associations were observed between serum total cholesterol and ARM incidence (see Table 3.3). HDL cholesterol was positively associated with ARM. One SD increase in HDL cholesterol was associated with a 20% increase in risk, adjusted for age and gender. Additional adjustment for atherosclerosis and APOE genotype only slightly decreased the estimates, indicating that these factors do not materially explain the association between HDL cholesterol and ARM.

To evaluate this finding in light of the relation between ARM and APOE, we tested the association across APOE genotypes. As expected, the risk of ARM was lower for ε4 carriers compared with the E3E3 genotype (OR 0.67, 95% CI 0.49-0.89). Figure 3.3 shows that the association between HDL cholesterol and ARM is consistent in APOE*3 and APOE*4 genotypes, but is strongest in the latter (*P*<.05). The inverse association in APOE*2 genotype was not statistically significant and may be due to chance. Since elevated HDL

cholesterol may be associated with a lower cardiovascular mortality and thereby explain its relationship with ARM, we also performed analyses in persons with a history of cardiovascular disease or diabetes mellitus at baseline. The associations were not affected, neither were they changed after exclusion of persons using cholesterol-lowering medication (data not shown).

The nature of our findings is unknown. A problem in interpreting the results is that we do not know whether systemic levels of cholesterol reflect tissue specific effects. Nevertheless, our data confirmed a positive association between serum HDL cholesterol and ARM.[82, 111] Furthermore, we found that this association was strongest in APOE*4 carriers. These findings ask for confirmation in other populations.

3.4 Dietary intake of antioxidants and risk of age-related maculopathy: a prospective population-based study

Background: *Age-related maculopathy (ARM) is the leading cause of incurable blindness and visual impairment in industrialized countries. Its aetiology is unknown, but there is evidence that oxidative stress is implied. Recently, a randomised trial reported that high-dose supplementation with β-carotene, vitamins C and E, and zinc protected against progression of ARM. We investigated in an observational study whether dietary intake of the same or different antioxidant micronutrients could achieve a similar risk reduction.* ***Methods:*** *We prospectively studied the association between dietary intake of antioxidants and incident ARM in 4139 participants aged 55 years and older in the population-based Rotterdam Study. Intake was assessed by a semi-quantitative food frequency questionnaire and different antioxidant nutrients were analysed. We used logistic regression analysis to estimate odds ratios.* ***Findings:*** *After a mean follow-up of 5.3 years, 362 subjects were diagnosed with incident ARM. Increased dietary intake of vitamin E, iron, and zinc were associated with a lower risk of ARM. Odds ratios (OR) per SD increase of intake were 0.88 (95% CI 0.78-0.99), 0.83 (95% CI 0.74-0.93), and 0.85 (95% CI 0.76-0.96), respectively, adjusted for age, sex, body-mass index, smoking status, pack-years of smoking, systolic blood pressure, atherosclerosis, serum total cholesterol, and alcohol intake. Except for iron, these associations were confirmed when quartiles of intake were analysed. An above-median dietary intake of β-carotene, vitamins C and E, and zinc was associated with a 55% reduced risk of ARM. Exclusion of supplement users did not change the results.* ***Interpretation:*** *High dietary intake of vitamin E and zinc may reduce the risk of ARM in a general population of elderly persons.* ***Submitted for publication***

Age-related maculopathy (ARM) is a degenerative disease of the macula, the central part of the retina, and results in the inability to read, recognize faces, drive, and move freely. ARM is confined to the age-category above 50 years and its prevalence steeply increases after the age of 70. There is no long-lasting cure for the majority of patients, making ARM the leading cause of irreversible blindness and visual impairment among elderly in industrialized countries.[96] As the number of persons 60 years or over

worldwide will nearly triple by 2050,[1] ARM is a fast growing burden to public health.

The pathophysiology of ARM is still poorly understood, but like other age-related disorders oxidative stress has been implicated. The retina seems particularly susceptible to oxidative stress because of its high concentration of oxygen, polyunsaturated fatty acids, and photosensitizers, in combination with an intense exposure to light.[19] To evaluate the relationship between oxidative stress and ARM in humans, epidemiological studies have investigated both dietary intake and serum levels of antioxidant vitamins, but results have been conflicting.[64, 170, 185, 205] Recent evidence supporting an association was provided by a trial of the Age-Related Eye Disease Study (AREDS) research group.[8] This is a randomised placebo-controlled clinical trial in which subjects with early ARM were assigned to receive supplements containing five to 13 times the recommended daily dose of β-carotene, vitamins C and E, and zinc. The study showed a statistically significant reduction from 31% to 23% in the six-year risk of progression to end-stage ARM. Critics of the study pointed at the high percentage of participants using supplements by themselves independent of randomisation, the safety risks of the high doses of supplements, in particular of zinc, and the subgroup analysis in retrospect.[10, 14, 18, 63, 174] Also, lutein and zeaxanthin, two caretenoids that are concentrated in the macula, were not investigated because they were not readily available at the initiation of the trial.[8]

The objective of our study was to test whether a combination of antioxidant nutrients similar to that used in the trial could achieve a protective effect when consumed as part of a normal diet instead of by supplementation. Another important issue is whether progression of ARM can be prevented at an earlier stage, considering the recent finding that drusen, the hallmark of early ARM, show oxidative protein modifications.[42] Therefore, we investigated the association between dietary intake of antioxidant nutrients, including the combination of nutrients previously described, and the incidence of early and late ARM in a large population-based cohort with five years of follow-up.

METHODS

Study Population

Information on the identification and description of the baseline study population has appeared in previous reports.[209] Briefly, the Rotterdam Study is a population-based prospective cohort study of the frequency and determinants of common cardiovascular, locomotor, neurological, and ophthalmologic diseases.[74] 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 ARM

A detailed description of the diagnostic procedures has been presented elsewhere.[209] Participants underwent a full eye examination, including stereo 35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) following pharmacological mydriasis. The resulting transparencies were graded with 12.5x magnification according to the International Classification and Grading System for ARM and AMD.[24] In this system, all ARM fundus signs within a standard circle (diameter 6000 μm) around the macular are recorded. Two well-trained graders, 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 drusen type, 0.80 for hyper pigmentation, and 0.58 for hypo pigmentation.

ARM was defined as the presence of either large (≥63 μm) soft distinct drusen with pigment irregularities, or indistinct (≥125 μm) or reticular drusen, or atrophic or neovascular ARM. Atrophic ARM was defined as any sharply demarcated round or oval area of apparent absence of the retinal pigment epithelium (RPE), larger than 175 μm, irrespective of location but within the grid, with visible choroidal vessels and no neovascular ARM. Neovascular ARM was defined as the presence of a serous or a hemorrhagic RPE detachment or a sub retinal neovascular membrane or a sub retinal haemorrhage, 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 ARM, were excluded from ARM classification. 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.

Dietary assessment

Dietary intake was assessed at baseline by means of a two-stage protocol. First, participants completed at home a checklist with foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on dietary habits, use of supplements, and prescribed diets. Next, participants underwent a standardized interview based on the checklist during their visit to the research centre. The interview was performed by a dietician, who used a validated, semi-quantitative food frequency questionnaire (SFFQ).[128] The SFFQ data were converted to total energy intake and nutrient intake using the computerized Dutch Food Composition Table.[41] For the

Characteristic	Mean (SD)	
Age, y	66.5	(7.2)
Sex, female (%)	2449	(59.2)
Body mass index, kg/m^2	26.3	(3.6)
Smoking status (%)		
Never	1375	(33.4)
Former	1802	(43.7)
Current	943	(22.9)
Median pack-years of smoking (inter-quartile range)		
Former smokers	18	(3-33)
Current smokers	30	(17-43)
Systolic blood pressure, mm Hg	137.4	(21.4)
Total cholesterol, mmol/l	6.7	(1.2)
Atherosclerosis score*	2.5	(1.1)
Alcohol intake, gram/day	10.6	(15.3)
Number of antioxidant supplement users (%)	556	(13.4)

* Information on the atherosclerosis composite score is presented in the Methods section

Table 3.4.1 *Baseline characteristics of the study sample (n=4139).*

current study, we selected the carotenoids α- and β-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, the vitamins A (retinol equivalents), C and E, and the cofactors for antioxidant enzymes copper, iron, selenium, and zinc.

Assessment of confounders

Information on all potential confounders was collected at baseline. During a home interview, participants were asked for their smoking habits, categorized as current, former, or never. At the study centre, height and weight were measured and body-mass index (weight divided by height squared) was calculated. Serum total cholesterol level was measured in non-fasting blood samples by means of an automated enzymatic procedure. Blood pressure was measured in sitting position at the right brachial artery with a random zero sphygmomanometer. Presence of sub-clinical atherosclerosis was determined by four different measurements: ankle-arm index, carotid wall thickness, atherosclerotic plaques in the carotid arteries, and calcifications in the abdominal aorta. 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. The lowest ratio of both legs was used. Carotid intima-media thickness and atherosclerotic plaques were assessed by means of ultrasonography. Aortic calcifications were detected on lateral radiographic films of the lumbar spine.

Finally, an atherosclerosis composite score was constructed by summing up points for the population-based deciles of carotid wall thickness and ankle-arm index, and points for the categories of carotid plaques and aortic calcifications. The composite score ranged from one to four. A detailed description of this score is provided elsewhere.[203] Alcohol intake was part of the dietary assessment and was expressed as grams per day. Persons who reported taking supplements containing carotenoids, vitamins A, C, or E, copper, iron, selenium, or zinc, as well as multivitamins or multiminerals, were classified as supplement users.

Study sample

Of the 6780 participants in the baseline ophthalmic examination, 6477 subjects underwent fundus photography and 6418 had gradable transparencies. Prevalent ARM was diagnosed in 582 subjects (9.1%), resulting in 5836 subjects at risk of ARM. Of this cohort, subjects were excluded from dietary assessment for two reasons. Dietary intake was not assessed in subjects (n=227) who had a decreased cognitive function (defined as a Cambridge Examination of Mental Disorders in the Elderly score <80), because they might provide unreliable answers regarding their food patterns. We also excluded nursing home residents (n=179) because their food was prepared by nursing home staff and would not reflect dietary habits in the past. Of the 5430 subjects eligible for dietary assessment, reliable dietary data were missing in 665 because of logical inconsistencies in dietary interviews, refusal to the second baseline visit when the food-frequency questionnaire was administered, or various logistic reasons. Eligible subjects without dietary data were on average somewhat older (1.7 years) compared with subjects with data, and included fewer women (7.4%). Other baseline characteristics were largely similar across the two groups.

In the cohort with dietary data (n=4765), 186 subjects died before the first follow-up examination and another 767 before the second follow-up. Of those alive at the first follow-up (n=4579), 13 subjects were lost to follow-up, 624 refused to participate and 14 had upgradeable photographs. Of those alive at the second follow-up (n=3812), 58 subjects were lost to follow-up, 666 refused to participate and 27 had upgradeable photographs. The study sample consists of 4139 subjects who had normal cognition, lived independently, had reliable dietary assessment, and participated in at least one follow-up examination.

Data analysis

Before studying its association with ARM, we adjusted the dietary intake of antioxidant nutrients for the total energy intake by means of the residual method described by Willett.[224] For each nutrient, linear regression analysis was performed with antioxidant intake as the dependent and total energy intake as the independent variable. This regression equation was used to calculate the expected mean antioxidant intake of the study population for the mean total

Nutrient	Mean daily intake (SD)	Odds ratio per SD (95% CI)*
Carotenoids		
α-Carotene	1.2 (0.8)	0.92 (0.79, 1.06)
β-Carotene	4.0 (2.2)	0.92 (0.80, 1.06)
β-Cryptoxanthin	0.3 (0.2)	0.97 (0.83, 1.12)
Lutein	2.3 (1.1)	0.96 (0.84, 1.10)
Zeaxanthin	0.1 (0.05)	0.97 (0.86, 1.10)
Vitamins		
Vitamin A (retinol equivalents)	0.8 (0.4)	0.95 (0.84, 1.08)
Vitamin C	120.5 (52.6)	0.98 (0.88, 1.10)
Vitamin E	13.8 (5.2)	0.88 (0.78, 0.99)
Trace elements		
Copper	1.2 (0.5)	0.91 (0.78, 1.06)
Iron	12.0 (2.2)	0.83 (0.74, 0.93)
Selenium	32.8 (8.4)	0.94 (0.83, 1.05)
Zinc	10.6 (2.0)	0.85 (0.76, 0.96)

*Adjusted for age, sex, follow-up time, body-mass index, smoking status, pack-years of smoking, systolic blood pressure, atherosclerosis, serum total cholesterol, and alcohol intake.

Table 3.4.2 *Mean energy-adjusted dietary intake per day of antioxidant nutrients in the study sample (n=4139), and odds ratios of incident age-related maculopathy per standard deviation (SD) increase of intake.*

energy intake of the study population. Next, for each individual the energy-adjusted intake was calculated by adding the expected mean antioxidant intake of the study population to the residual derived from the regression analysis.

We estimated the risk of ARM associated with the dietary intake of antioxidant nutrients at baseline with logistic regression analysis adjusted for time of follow-up. Intake of each nutrient was entered into the model either as a linear term or as dummy variables representing the three highest quartiles. In the first case, the regression coefficient was expressed per standard deviation (SD) increase. In the second case, quartiles were analysed both as a categorical variable and as a continuous variable to test for a trend. Quartiles and SDs were based on the distribution within the study sample. We adjusted in all analyses for age, gender, body mass index, smoking status, pack-years of smoking, systolic blood pressure, serum total cholesterol, atherosclerosis, and alcohol intake. In the analysis of vitamin E, additional adjustment was made for intake of polyunsaturated fat, because of its strong link with this fat-soluble vitamin and its reported association with ARM.[173] Missing values of categorical variables were represented in the model by a missing indicator. For continuous variables, missing values were replaced by the mean or median of the study

sample, depending on the distribution. To distinguish between the effect of antioxidants from food and from supplements, all analyses were repeated after exclusion of supplement users at baseline.

We constructed a composite score of the combined intake of the nutrients as were investigated in the AREDS trial.[8] To secure large enough groups with a high or low intake of each of four nutrients, we used the median intake per nutrient, based on the total sample, as cut-off value. The high intake group consisted of persons with an above median intake of each of four nutrients. The low intake group had a below median intake of each nutrient, and all persons in between were considered the reference category.

Associations are presented as odds ratios (OR) with 95% confidence intervals (CI). All analyses were performed using SPSS for Windows, Release 11.0.1.2001 (SPSS Inc., Chicago, Illinois).

RESULTS

Baseline characteristics of the study sample are presented in Table 3.4.1. Mean age was 66.5 years, 59.2% was female, 22.9% was current smoker, and 13.4% used antioxidant supplements. Follow-up of participants was on average 5.2 years, with a range of 1.0 to 9.7 years (median 6.3 years). During this period, 362 subjects were diagnosed with incident ARM, of whom the majority had early ARM and 12 had late ARM (4 atrophic and 8 neovascular ARM). Incident cases with early ARM had either large soft drusen with pigment irregularities (n=261), or indistinct drusen without (n=109), or with pigment irregularities (n=35). One of the persons with incident late ARM developed this at the first follow-up examination and 13 at second. Six cases had soft distinct drusen or pigment 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 dietary intake (P=0.67, adjusted for age and gender).

Table 3.4.2 gives the mean dietary intake of the antioxidant nutrients in the study sample, adjusted for energy intake. The ORs of incident ARM per standard deviation (SD) increase in nutrient intake are also shown in this table. A statistically significant inverse association was observed for the intake of vitamin E, iron, and zinc. Adjusting for age, sex, body-mass index, smoking status, pack-years of smoking, systolic blood pressure, atherosclerosis, serum total cholesterol, and alcohol intake, one SD increase in vitamin E intake was associated with a 12% reduction in the risk of ARM (OR 0.88, 95% CI 0.78-0.99). The ORs per SD increase in intake of iron and zinc were 0.83 (95% CI 0.74-0.93) and 0.85 (95% CI 0.76-0.96), respectively.

The results of the analyses with quartiles of nutrient intake are graphically presented in Figure 3.4. For each nutrient the P-value of the test for trend is given. The highest compared to the lowest quartile of both vitamin E (P=.009) and zinc (P=.002) was associated with a reduced risk of ARM. The association between quartiles of iron intake and incident ARM was not statistically significant (P=.12).

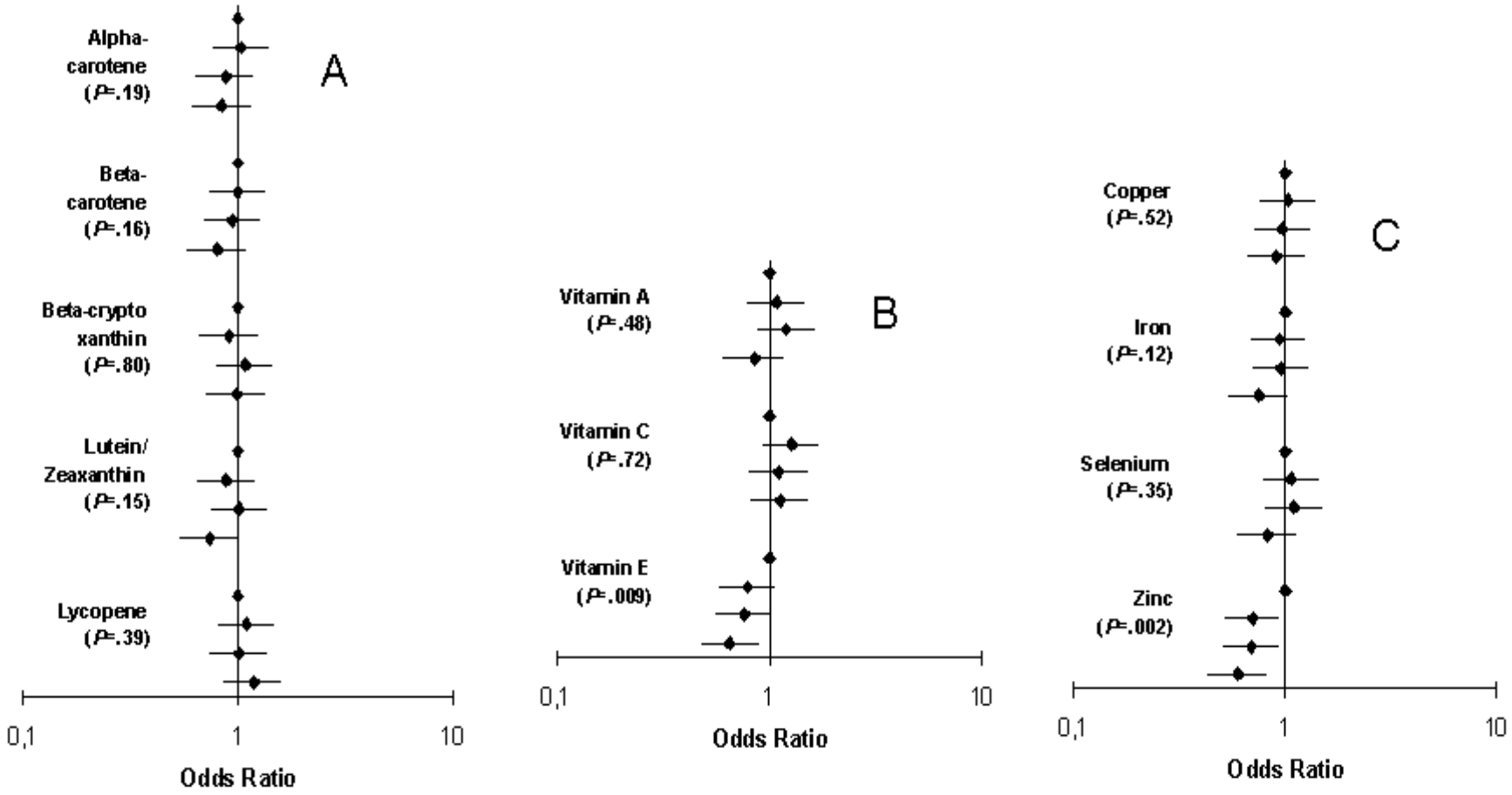

Alpha-carotene (P=.19)
Beta-carotene (P=.16)
Beta-crypto xanthin (P=.80)
Lutein/ Zeaxanthin (P=.15)
Lycopene (P=.39)
A
0,1
1
10
Odds Ratio
Vitamin A (P=.48)
Vitamin C (P=.72)
Vitamin E (P=.009)
B
0,1
1
10
Odds Ratio
Copper (P=.52)
Iron (P=.12)
Selenium (P=.35)
Zinc (P=.002)
C
0,1
1
10
Odds Ratio

Figure 3.4 *Odds ratios for incident age-related maculopathy according to quartiles of dietary intake of antioxidant carotenoids (A), vitamins (B), and trace elements (C), adjusted for age, sex, follow-up time, body-mass index, smoking status, pack-years of smoking, systolic blood pressure, atherosclerosis, serum total cholesterol, and alcohol intake. Lowest quartile (top) was considered the reference group. For each nutrient the P-value of the test for trend is given.*

Table 3.4.3 presents the impact of the combined dietary intake of four antioxidants that were defined by the AREDS trial on the incidence of ARM. An above-median intake of β-carotene, vitamins C and E, and zinc, compared with a below-median intake of at least one of these nutrients, was associated with a reduced risk of ARM (OR 0.44, 95% CI 0.26-0.77), adjusted for all potential confounders. In persons with a below-median intake of all four nutrients the risk of ARM was increased (OR 1.33, 95% CI 0.99-1.79).

The results were not substantially different when subjects that used antioxidant supplements at baseline were excluded. Also, adding supplement users to the highest quartile of dietary intake did not change the results.

DISCUSSION

In this observational study, we found that an increased dietary intake of vitamin E and zinc reduced the incidence of ARM in an elderly population. An above-median intake of the predefined combination of vitamins C and E, β-carotene, and zinc, was associated with a 55% lower risk of ARM.

In contrast to a case-control design, we assessed dietary intake before the onset of ARM. Therefore, information (recall) bias is not likely to be involved. Misclassification may still play a role since dietary assessment was performed only once. Also, questionnaires are subject to over- and underreporting, and dietary intake does not account for variation in digestion and absorption. Most likely, this misclassification is non-differential and will have lead to an underestimation of the true associations. Measurement of serum levels may offer a solution to the aforementioned problems. However, it is unclear whether one-time serum levels reflect long-term exposure rather than recent nutritional intake. Although dietary assessment was performed only once, a validation study of our questionnaire demonstrated reproducible and valid estimates.[128]

Exclusion of persons using antioxidant supplements at baseline did not change our results, suggesting that antioxidants from food are responsible for the observed associations. We also investigated the combined effect of antioxidants from food and from supplements, which resulted in similar risk estimates. The independent effect of antioxidant supplements on the risk of ARM could not be examined because of the small number of users in our population and the lack of data on duration and dosage of supplement use.

	Categories of intake*		
	Low	Middle (Reference)	High
Number (cases)	522 (63)	3260 (285)	357 (14)
Odds ratios (95% CI)			
Crude	1.47 (1.10, 1.96)	1.00	0.42 (0.24, 0.72)
Age and gender adjusted	1.36 (1.01, 1.83)	1.00	0.44 (0.25, 0.76)
Fully adjusted†	1.33 (0.99, 1.79)	1.00	0.44 (0.26, 0.77)

*Categories were defined by using the median intake per nutrient as cut-off value and classifying above-median intake of all selected nutrients into high, and below-median intake of all nutrients into low intake.
† Adjusted for age, sex, follow-up time, body-mass index, smoking status, pack-years of smoking, systolic blood pressure, atherosclerosis, serum total cholesterol, and alcohol intake.

Table 3.4.3 *Odds ratios of incident age-related maculopathy for the combined intake of four predefined antioxidant nutrients (vitamins C and E, β-carotene and zinc).*

Recent data suggest that oxidative protein modifications may have a critical role in the formation of drusen,[42] the hallmark of early ARM, implying that the strongest effect of antioxidants is expected at the early stage of the disease. In the AREDS trial, only late ARM was an endpoint in the analysis, and since the number of incident late ARM was very low in persons with early ARM at baseline, there was no evidence of a treatment benefit in this subgroup.[8] We studied a cohort free of ARM at baseline and our incident cases included mainly early disease. Exclusion of the 12 persons with incident late ARM did not change the results. We therefore conclude that antioxidants have an effect on the development of drusen and other signs of early ARM. Given the well-documented risk of early ARM to progress to the blinding stage of late ARM, it seems justified to assume that risk factors of early ARM also affect the risk of late ARM.[116, 200]

We studied all carotenoids and vitamins that have been implicated in the risk of ARM previously, as well as four trace elements that act as a cofactor of antioxidant enzymes relevant to the retina.[19] Many studies have investigated the relationship between antioxidants and ARM, but they varied in study design, nutrients included, and outcome definition. To our knowledge, two studies have investigated the relation between dietary vitamin E and ARM, of which only one included early ARM. In the Eye-disease Case-Control Study (EDCCS) persons with neovascular late ARM (n=356) did not have a lower dietary intake of vitamin E.[170] In contrast, the population-based Beaver Dam Eye Study reported a lower risk of separate signs of early ARM with higher intake of vitamin E from diet and supplements, of which only the association with large drusen reached statistical significance.[205] The size of this sample was less than half that of ours. The association between dietary intake of zinc and

early ARM was studied in two comparable prospective cohort studies. The Beaver Dam Eye Study observed some inverse associations, both cross-sectional and prospective, with separate signs of early ARM.[136] No evidence for a relationship was found in the Blue Mountains Eye Study.[56, 182] Two very large cohort studies assessed the incidence of self-reported late ARM and found no relation with zinc intake. The EDCCS reported a 40% reduced risk of neovascular late ARM with a high dietary intake of lutein and zeaxanthin.[170] Past intake of different carotenoids, but not lutein/zeaxanthin, was associated with incident early ARM in the BDES.[205] No relationship was seen in the BMES. To our knowledge, no epidemiological studies have previously investigated the effect of dietary copper, iron or selenium on the risk of ARM. Reports on serum levels of copper in monkeys[150] and selenium in humans[138] suggested lower concentrations in the presence of ARM, but these associations could not be confirmed in our study. Our finding that iron may protect against ARM has not been reported before.

Our data provide evidence that vitamin E and zinc protect against the early, pre-symptomatic, stage of the ARM. This suggests that prevention of ARM can be achieved by a high intake of dietary antioxidants. Our finding is in line with the results from the AREDS trial, but indicates that a protective effect may be achieved by dietary intake instead of supplement use, and at an earlier stage of the disease. Based on our results, persons should be encouraged to increase their consumption of food items containing vitamin E and zinc even before the early signs of ARM are present. Examples of substances containing vitamin E include vegetable oil, eggs and nuts, while wholemeal products and dairy products are suppliers of zinc. Persons above 55 years of age should be encouraged to increase their consumption of these food items to lower their risk of ARM.

3.5 Fat for the fundus: dietary fat and the risk of incident age-related maculopathy

Objective: *To examine the relationship between dietary intake of total and specific types of fat and the risk of incident age-related maculopathy (ARM).* ***Design:*** *Prospective population-based cohort study.* ***Participants:*** *The study sample consisted of 4139 persons aged 55 years and older without ARM at baseline, who participated in at least one follow-up examination after 2 and 6½ years. Dietary habits were assessed using a food frequency questionnaire. After a mean follow-up of 5.2 years, 362 subjects were diagnosed with incident ARM.* ***Main Outcome Measure:*** *Incidence of ARM.* ***Results:*** *We found no associations between incident ARM and the intake of total fat, cholesterol, saturated fat, trans fat, monounsaturated fat, polyunsaturated fat, or n-3 polyunsaturated fat. Higher intake of n-6 polyunsaturated fatty acids was associated with an increased risk of ARM (P for trend 0.4), adjusted for age and gender. However, after additional adjustments including vitamin E, this association disappeared. Frequency of fish intake did not affect the incidence of ARM in our cohort. The associations were similar in participants without cardiovascular disease or diabetes mellitus.* ***Conclusion:*** *Dietary intake of total fat or specific types of fat was not associated with incident early ARM in this population-based cohort.* ***Submitted for publication***

Age-related maculopathy (ARM) is the most common cause of incurable blindness among elderly in industrialized countries and prevention of this disease could have a large impact on the aging population.[96, 114] Unfortunately, very few modifiable risk factors are presently known.[54] Although an association with some cardiovascular risk factors, in particular smoking, has been demonstrated, no definite evidence for a vascular origin of ARM has been put forward yet.[186] To further test this hypothesis, a few studies have investigated dietary intake of fat in relation to ARM.[36, 72, 135, 173, 183] Subtypes of fat that are known to increase the risk of coronary heart disease, such as saturated fat and *trans* unsaturated fatty acids, may also be linked to AMD.[79] On the other hand, polyunsaturated fatty acids (PUFAs) and fish were shown to protect against cardiovascular disease and may be inversely associated with ARM.[79, 129]

The high concentration of n-3 PUFAs in the human retina is another reason to suspect a relationship between fat intake and ARM.[88, 199] Because the precursors of these fatty acids cannot be synthesized endogenously, dietary intake determines their blood levels and will have an impact on retinal development and function.[193] In addition, PUFAs are highly susceptible to oxidative damage and their concentration may affect the level of oxidative stress, a presumed cause of ARM.[19] Furthermore, fatty acids may modulate the immune response.[176] With the growing interest in the inflammatory processes in ARM, this mechanism provides another potential link between fat intake and ARM.[66]

Results from previous studies suggest that a diet high in n-3 PUFAs and fish is associated with a lower risk of ARM.[36, 173, 183] Findings concerning other types of fat were inconsistent.[36, 72, 135, 173, 183] Most previous studies used late stage ARM as outcome measure or employed a case-control design. We therefore examined the association between dietary intake of total and specific types of fat and incident early ARM in a population-based cohort study.

METHODS

Population

Information on the identification and description of the baseline study population has appeared in previous reports.[209] Briefly, the Rotterdam Study is a population-based prospective cohort study of the frequency and determinants of common cardiovascular, locomotor, neurological, and ophthalmologic diseases.[74] 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.[209] Participants underwent a full eye examination, including stereo 35° fundus photography (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) centred 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 its end-stages age-related macular degeneration (AMD).[24] 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 hyper-pigmentation, and 0.58 for hypo-pigmentation.

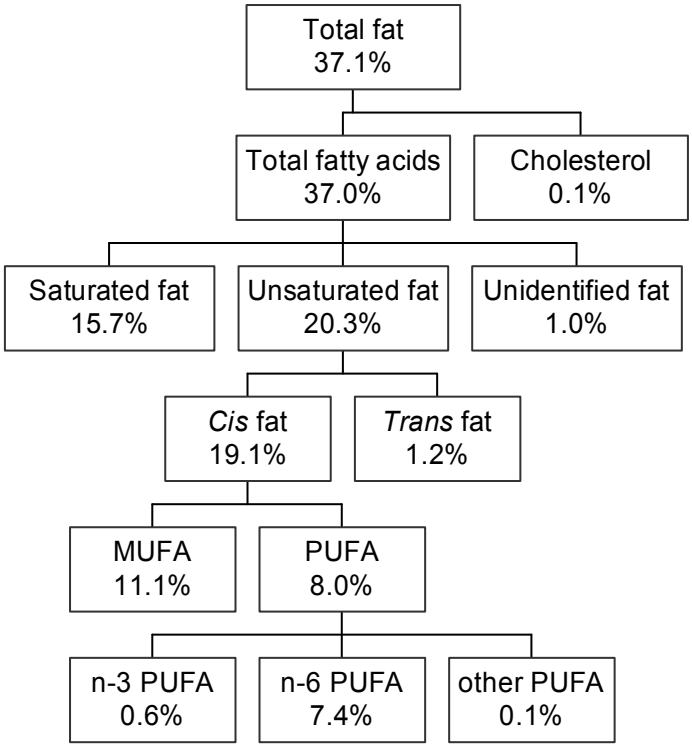
ARM was defined as the presence of either large (≥63 μm) soft distinct drusen with pigment irregularities, or indistinct (≥125 μm) or reticular drusen, or atrophic or neovascular AMD. Atrophic AMD was defined as any sharply demarcated round or oval area of apparent absent retinal pigment epithelium, larger than 175 μm, irrespective of location but within the grid, with visible choroidal vessels and no neovascular AMD. Neovascular AMD was defined as the presence of a serous or a hemorrhagic 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. 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.

Dietary assessment

Dietary intake was assessed at baseline by means of a two-stage protocol. First, participants completed at home a checklist with foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on dietary habits, use of supplements, and prescribed diets. Next, participants underwent a standardized interview based on the checklist during their visit to the research centre. The interview was performed by a dietician, who used a validated, semi-quantitative food frequency questionnaire (SFFQ).[128] The SFFQ data were converted to total energy intake and nutrient intake using the computerized Dutch Food Composition Table.[41] For the current study, we selected intake of total fat, cholesterol, saturated fat, *trans* fat, *cis* monounsaturated fatty acids (MUFA), *cis* polyunsaturated fatty acids (PUFA), n-6 PUFA, and n-3 PUFA.

Assessment of confounders

Information on all potential confounders was collected at baseline. During a home interview, participants were asked for their smoking habits, categorized as current, former, or never. At the study centre, height and weight were measured and body-mass index (weight divided by height squared) was calculated. Blood pressure was measured in sitting position at the right brachial artery with a random zero sphygmomanometer. As a measure of atherosclerosis, we used a composite score based on four different measurements: ankle-arm index, carotid wall thickness, atherosclerotic plaques



*MUFA indicates monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Figure 3.5 *Mean fat intake of the study sample as percentage of total energy intake.*

in the carotid arteries, and calcifications in the abdominal aorta. In short, 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. The lowest ratio of both legs was used. Carotid intima-media thickness and atherosclerotic plaques were assessed by means of ultrasonography. Aortic calcifications were detected on lateral radiographic films of the lumbar spine. An atherosclerosis composite score was constructed by summing up points for the population-based deciles of carotid wall thickness and ankle-arm index, and points for the categories of carotid plaques and aortic calcifications. The composite score ranged from one to four. A detailed description of this score is provided elsewhere (Van Leeuwen et al, IOVS in press).

Study sample

At the baseline phase of the study, 6477 participants underwent fundus photography and 6418 subjects (99.1%) had gradable transparencies. Prevalent ARM was diagnosed in 582 subjects (9.1%), including 106 cases of AMD, resulting in a cohort of 5836 subjects free of ARM. Of this cohort, subjects were excluded from dietary assessment for two reasons. Dietary intake was not assessed in subjects (n=227) who had a decreased cognitive function (defined as a Cambridge Examination of Mental Disorders in the Elderly score <80), because they might provide unreliable answers regarding their food patterns.

We also excluded nursing home residents (n=179) because their food was prepared by nursing home staff and would not reflect dietary habits in the past. Of the 5430 subjects eligible for dietary assessment, reliable dietary data were missing in 665 because of logical inconsistencies in dietary interviews, refusal to the second baseline visit when the food-frequency questionnaire was administered, or various logistic reasons.

In the cohort with complete dietary data (n=4765), 156 (3.3%) subjects died before the first follow-up examination and another 505 (10.6%) before the second follow-up. Of those alive at the first screening (n=4609), 673 (14.6%) subjects were either lost to follow-up, refused to participate or had ungradable photographs. Of those alive at the second follow-up (n=4104), 1043 (25.4%) subjects were lost to follow-up, refused to participate or had ungradable photographs. Thus, the study sample consists of 4139 subjects who had normal cognition, lived independently, had reliable dietary assessment, and participated in at least one follow-up examination. The composite score of atherosclerosis was missing in 526 persons.

Statistical analysis

Because of the strong correlation between fat intake and total energy intake, we calculated energy-adjusted intake of fat by means of the residual method described by Willett.[224] For each fat subtype, linear regression analysis was performed with fat intake as the dependent and total energy intake as the independent variable. This regression equation was used to calculate the expected mean fat intake of the study population for the mean total energy intake of the study population. Next, for each individual the energy-adjusted intake was calculated by adding the expected mean fat intake of the study population to the residual derived from the regression analysis.

The association between dietary intake of fat subtypes at baseline and incident ARM was analysed with logistic regression adjusted for age, gender, and time of follow-up. Quartiles of fat intake were entered into the model either as a categorical or as a continuous variable to test for a trend. Quartiles were based on the distribution in the study sample. Additional adjustments were made for smoking status, pack-years of smoking, body mass index, and alcohol intake. Since vitamin E was shown to be associated with a decreased risk of ARM and vitamin E is fat-soluble and consumed with fat, we additionally adjusted for vitamin E.[184]

We performed an additional restricted analysis in participants without cardiovascular disease (myocardial infarction or stroke) or diabetes mellitus at baseline or during follow-up, because these conditions may result in a change in dietary habits.

To test whether the incidence of ARM was different in the eligible population with missing dietary data as compared to the study sample, we used logistic regression analysis adjusted for age, gender, and follow-up time. Missing values of categorical variables were represented in the model by a missing indicator. For continuous variables, missing values were replaced by

Characteristic	Mean (SD)	
Age, y	66.5	(7.2)
Sex, female (%)	2449	(59.2)
Body mass index, kg/m^2	26.3	(3.6)
Smoking status (%)		
Never	1375	(33.4)
Former	1802	(43.7)
Current	943	(22.9)
Systolic blood pressure, mm Hg	137.4	(21.4)
Atherosclerosis score†	2.5	(1.1)
Alcohol intake, g/d	10.6	(15.3)
Vitamin E intake, mg	13.8	(6.1)
Total energy intake, kJ	8272	(2106)
Energy-adjusted intake of		
Total fat, g/d	80.6	(13.3)
Cholesterol, g/d	0.2	(0.061)
Saturated fat, g/d	34.2	(7.0)
Trans fat, g/d	2.6	(1.0)
MUFA, g/d	24.3	(5.3)
PUFA, g/d	17.5	(6.6)
n-6 PUFA, g/d	16.1	(6.5)
n-3 PUFA, g/d	1.3	(0.6)

*MUFA indicates monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
†Information on the atherosclerosis composite score is presented in the Methods section.

Table 3.5.1 *Baseline characteristics of the study sample (n=4139).**

the mean or median of the study sample, depending on the distribution. All associations are presented as odds ratios, which can be interpreted as relative risks, with 95% confidence intervals (CI). All analyses were performed using SPSS for Windows, Release 11.0.1.2001 (SPSS Inc., Chicago, Illinois).

RESULTS

The baseline characteristics of the study sample are presented in Table 1. Mean age was 66.5 years, 59.2% was female, and mean daily energy intake was 8272 kJ (1977 kcal). The average consumption of total fat was 81 grams per day, which constituted 37.1% of the total energy intake. The Figure presents the contribution of fat and its subtypes to total energy intake for the study sample.

Follow-up of participants was on average 5.2 years, with a range of 1.0 to 9.7 years (median 6.3 years). During this period, 362 subjects were diagnosed with incident ARM, of whom the majority had early ARM and 12 had AMD (four atrophic and eight neovascular AMD). Incident cases with early ARM had either large soft drusen with pigment irregularities (n=261), or indistinct drusen without (n=109), or with pigment irregularities (n=35). One of the persons with incident late ARM developed this at the first follow-up examination and 13 at second. Six cases had soft distinct drusen or pigment 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 dietary intake (*P*=0.67, adjusted for age and gender).

The association between dietary intake of fat subtypes and incident ARM is presented in Tables 2 and 3. No associations were observed for the intake of total fat, cholesterol, saturated fat, MUFA, and n-3 PUFA. Persons in the third compared to the first quartile of *trans* fat intake had a statistically significant increased risk of ARM when adjusting for age and gender (OR 1.56, 95% CI 1.14-2.15). This association remained when additional adjustments were made for body-mass index, smoking status, pack-years of smoking, systolic blood pressure, and alcohol intake. However, when an atherosclerosis composite score was added to the model, the association disappeared (OR 1.09, 95% CI 0.74-1.60).

Adjusted for age and gender, we found an inverse association with ARM for a higher intake of PUFA (OR third vs. first quartile 0.73, 95% CI 0.53-0.99). The association disappeared with additional adjustment for other confounders. However, with additional adjustments, there was still a protective effect of n-6 PUFA intake (OR highest compared with lowest quartile 0.73, 95% CI 0.54-1.00, *P* for trend .04). When we additionally adjusted for vitamin E intake, the association with n-6 PUFA disappeared (OR 0.84, 95% CI 0.55-1.29).

In Table 4 we present the association between dietary fish intake and incident ARM. Consumption of fish more than once per week versus less than once per month was inversely associated with incident ARM, but this association was not statistically significant (OR 0.85, 95% CI 0.52-1.37).

Similar results were obtained when we repeated the analyses in persons who did not use lipid-lowering medication, either at baseline or at follow-up (data not shown). Excluding participants with cardiovascular disease or diabetes mellitus at baseline or during follow-up also resulted in similar risk estimates, except that the association between saturated fat and ARM was stronger. The OR of the highest compared with the lowest quartile of saturated fat intake changed from 1.24 (95% CI 0.91-1.69) to 1.40 (95% CI 0.98-2.01), adjusting for age and gender. However, additional adjustment for atherosclerosis resulted in a similar disappearance.

Type of fat	Quartiles				*P* for trend
	1st	2nd	3rd	4th	
Total fat					
No. of cases (%)	95 (9.2)	86 (8.3)	85 (8.2)	96 (9.3)	
Median intake, g/d	66.4	76.7	84.0	95.0	
Age- and sex-adjusted OR (95% CI)	1.0	0.93 (0.68, 1.27)	0.91 (0.66, 1.24)	1.02 (0.75, 1.38)	.95
Multivariate OR (95% CI)*	1.0	0.94 (0.68, 1.28)	0.92 (0.67, 1.27)	1.09 (0.79, 1.50)	.65
Cholesterol					
No. of cases (%)	81 (7.8)	98 (9.5)	87 (8.4)	96 (9.3)	
Median intake, g/d	0.17	0.21	0.25	0.30	
Age- and sex-adjusted OR (95% CI)	1.0	1.23 (0.90, 1.69)	1.09 (0.79, 1.50)	1.19 (0.87, 1.64)	.44
Multivariate OR (95% CI)*	1.0	1.22 (0.87, 1.70)	1.12 (0.80, 1.58)	1.12 (0.80, 1.58)	.66
Saturated fat					
No. of cases (%)	83 (8.0)	77 (7.4)	98 (9.5)	104 (10.1)	
Median intake, g/d	27.0	31.8	35.6	42.1	
Age- and sex-adjusted OR (95% CI)	1.0	0.93 (0.67, 1.30)	1.20 (0.88, 1.64)	1.24 (0.91, 1.69)	.08
Multivariate OR (95% CI)*	1.0	0.92 (0.66, 1.29)	1.13 (0.82, 1.56)	1.11 (0.81, 1.54)	.32
Trans fat					
No. of cases (%)	76 (7.4)	81 (7.8)	112 (10.8)	93 (9.0)	
Median intake, g/d	1.7	2.3	2.8	3.6	
Age- and sex-adjusted OR (95% CI)	1.0	1.09 (0.78, 1.52)	1.56 (1.14, 2.15)	1.13 (0.81, 1.56)	.19
Multivariate OR (95% CI)*	1.0	1.03 (0.73, 1.45)	1.38 (0.99, 1.94)	1.00 (0.70, 1.41)	.75

*Adjusted for body-mass index, smoking status, pack-years of smoking, systolic blood pressure, alcohol intake, and vitamin E intake

Table 3.5.2 *Association between incident age-related maculopathy and dietary intake of total fat and subtypes of fat.*

Type of fat	Quartiles				*P* for trend
	1st	2nd	3rd	4th	
MUFA					
No. of cases (%)	91 (8.8)	86 (8.3)	91 (8.8)	94 (9.1)	
Median intake, g/d	19.0	22.5	25.3	29.9	
Age- and sex-adjusted OR (95% CI)	1.0	0.98 (0.72, 1.35)	1.09 (0.80, 1.49)	1.14 (0.83, 1.55)	.32
Multivariate OR (95% CI)*	1.0	0.96 (0.70, 1.32)	1.07 (0.78, 1.47)	1.15 (0.84, 1.58)	.30
PUFA					
No. of cases (%)	105 (10.2)	94 (9.1)	78 (7.5)	85 (8.2)	
Median intake, g/d	10.6	15.1	19.0	24.8	
Age- and sex-adjusted OR (95% CI)	1.0	0.90 (0.67, 1.21)	0.73 (0.53, 0.99)	0.80 (0.59, 1.08)	.07
Multivariate OR (95% CI)*	1.0	1.01 (0.73, 1.40)	0.86 (0.59, 1.25)	1.06 (0.65, 1.73)	.88
n-6 PUFA					
No. of cases (%)	109 (10.5)	87 (8.4)	83 (8.0)	83 (8.0)	
Median intake, g/d	9.3	13.7	17.5	23.3	
Age- and sex-adjusted OR (95% CI)	1.0	0.80 (0.59, 1.08)	0.76 (0.56, 1.03)	0.74 (0.54, 1.00)	.04
Multivariate OR (95% CI) excluding vitamin E*	1.0	0.81 (0.60, 1.09)	0.78 (0.58, 1.06)	0.74 (0.54, 1.00)	.06
Multivariate OR (95% CI) including vitamin E*	1.0	0.86 (0.62, 1.20)	0.88 (0.61, 1.28)	0.92 (0.56, 1.51)	.71
n-3 PUFA					
No. of cases (%)	95 (9.2)	95 (9.2)	88 (8.5)	84 (8.1)	
Median intake, g/d	0.8	1.1	1.4	2.0	
Age- and sex-adjusted OR (95% CI)	1.0	1.06 (0.78, 1.44)	0.99 (0.73, 1.35)	0.93 (0.68, 1.27)	.56
Multivariate OR (95% CI)*	1.0	1.04 (0.76, 1.41)	0.99 (0.72, 1.35)	0.93 (0.68, 1.28)	.61

*Adjusted for body-mass index, smoking status, pack-years of smoking, systolic blood pressure, alcohol intake, and vitamin E intake

Table 3.5.3 *Association between incident age-related maculopathy and dietary intake of subtypes of fat.*

	Frequency of fish intake (times per week or month)				*P* for trend
	<1/mo	≥1/mo - <1/wk	1/wk	>1/wk	
No. of cases (%)	107 (8.6)	119 (9.0)	114 (9.0)	22 (6.8)	
Median intake, g/d	0	9.9	28.5	49.0	
Age- and sex-adjusted OR (95% CI)	1.0	1.04 (0.79, 1.38)	1.07 (0.81, 1.42)	0.85 (0.52, 1.37)	.93
Multivariate OR (95% CI)*	1.0	1.07 (0.81, 1.42)	1.10 (0.83, 1.46)	0.86 (0.53, 1.39)	.98

*Adjusted for body-mass index, smoking status, pack-years of smoking, systolic blood pressure, alcohol intake, and vitamin E intake.

Table 3.5.4 *Association between incident age-related maculopathy and dietary intake of fish.*

DISCUSSION

Data from this population-based prospective cohort study did not reveal strong or consistent associations between dietary intake of total fat or its subtypes and incident ARM. A borderline statistically significant inverse association between n-6 PUFA and ARM disappeared after additional adjustment for vitamin E intake.

In the Beaver Dam Eye Study, high intake of saturated fat and cholesterol was associated with an increased risk of early ARM.[135] In a combined analysis of the Nurses' Health Study and Health Professionals Follow-up Study, total fat and linolenic acid were positively associated with risk of late ARM.[36] This was unexpected, since linolenic acid was inversely related to cardiovascular disease risk in the same study populations.[17, 78] The Eye Disease Case-Control Study reported that higher intake of vegetable fat, MUFA, PUFA, and linoleic acid may be associated with a greater risk of neovascular AMD.[135] The two latter studies, as well as the Blue Mountains Eye Study, observed an inverse association between fish intake and late ARM. So, previous studies on dietary fat intake and ARM have been inconsistent in their results, except for the notion that fish intake may protect against ARM. Possible reasons for the discrepancies include different case definitions, recall bias, and chance findings.

In contrast to a case-control design, we assessed dietary intake before the onset of ARM. Therefore, information (recall) bias is less likely to be involved. Misclassification may still play a role since questionnaires are subject to over- and underreporting, and dietary intake does not account for variation in digestion and absorption. Most likely, this misclassification is non-differential and will have lead to an underestimation of the true association. Although dietary assessment was performed only once, a validation study of our questionnaire demonstrated reliable and valid estimates.[128] Misclassification was further reduced when we excluded participants with cardiovascular disease and diabetes, because these conditions may result in a change in dietary habits. This restricted analysis showed overall similar results.

As summarized in the introduction, there are many possible links between fat intake and ARM. One of these links is atherosclerosis, which is promoted by specific types of fat, such as saturated fat and *trans* unsaturated fat,[79] and which in itself may be a determinant of ARM.[208] We observed a non-significant trend of an increased risk of ARM with higher intake of saturated fat (P=.08, adjusted for age and gender). However, this association disappeared after further adjustment for other potential confounders. Overall, our data do not support the hypothesis that dietary fats are similarly related to both ARM and cardiovascular disease.

Oxidative stress provides another potential link between fat intake and ARM. PUFAs are susceptible to oxidation and produce oxygen radicals. On the other hand, consumption of fatty acids is correlated with intake of fat-soluble vitamins, including vitamin E. This antioxidant has been shown to protect against ARM progression. We found that intake of PUFAs showed an inverse

association with ARM incidence, but that this association diminished after additional adjustment for vitamin E. This suggests that vitamin E acts as a confounder in the association between PUFA intake and ARM, providing further evidence for a protective effect of vitamin E. Long-chain n-3 PUFAs, especially docosahexaenoic acid (DHA), are an important constituent of photoreceptor outer segments, and dietary intake of these fatty acids is required for their constant renewal. Deficiency of n-3 PUFAs in animals, in combination with light exposure, was found to induce retinal dysfunction.[193] Fish is the main supplier of DHA and may therefore promote retinal function and possibly influence the risk of ARM. We found only a non-significant protective effect of fish intake, while three other studies have reported stronger effect. The reason for our negative finding may be the infrequent consumption of fish in our population, with only 6% of participants reporting a fish intake of more than once per week. For comparison, this percentage was approximately 20-30% in two US study cohorts.[70, 80]

In conclusion, in this population of Dutch elderly persons, no association was observed between dietary intake of total fat or specific types of fat and the incidence of ARM.

3.6 Is medication use associated with the incidence of early age-related maculopathy? Pooled findings from three continents

Objective: *To investigate whether there is an association between the use of medication and the incidence of early age-related maculopathy (ARM).* ***Design:*** *Pooled data from three prospective, population-based cohort studies.* ***Participants:*** *Subjects without early and late ARM at baseline who participated in the follow-up of the Beaver Dam Eye Study (n=3012), the Rotterdam Study (n=3434), and the Blue Mountains Eye Study (n=2203).* ***Methods:*** *Stereoscopic colour fundus photographs of all participants were graded according to a standardized protocol. At baseline, current use of prescription and over-the-counter medication was assessed by interview while the drug name was confirmed at the research centres. Procedures and definitions were similar at both baseline and follow-up across the three study sites.* ***Main outcome measures:*** *Incidence of early ARM, defined as the presence at follow-up of either soft distinct drusen with pigment changes, or soft indistinct, or reticular drusen.* ***Results:*** *In the pooled cohort, 53.3% of participants used at least one of the medications selected for this study. Within a mean period of 5.6 years, a total of 683 subjects developed early ARM. Users of antihypertensive medication in general, and beta-blockers in particular, had a borderline statistically significant increased risk of early ARM (Odds Ratio (OR) for beta-blockers 1.3; 95% Confidence Interval (CI) 1.0, 1.6) when adjusted for systolic (or diastolic) blood pressure and other confounders. A protective effect of borderline significance was found among women using hormone replacement therapy (OR 0.6; 95% CI 0.4, 1.0) and in persons using tricyclic antidepressants (OR 0.4; 95% CI 0.2, 1.0). In contrast with beta-blockers, the direction and magnitude of the association with hormone replacement therapy and tricyclic antidepressants was inconsistent among the three study populations.* ***Conclusions:*** *Pooled data from three population-based studies showed no strong associations between medication use and the incidence of early ARM. Of borderline significance were a slightly increased risk among users of beta-blockers and a reduced risk among users of hormone replacement therapy and users of tricyclic antidepressants. Although beta-blocker use could be a proxy for systemic hypertension, these findings warrant further investigations, preferably including information on the dosage and duration of drug exposure.* ***Submitted for publication***

Age-related maculopathy (ARM) is a progressive, degenerative disorder of the retina, and its pathogenesis is poorly understood. Many potential risk factors have been examined, but for most the results

were negative or inconsistent.[54] Only smoking, hypertension, and the intake of antioxidants have been identified as risk factors that can be modified.[5, 8, 127, 184]

It is thought that the retinal pigment epithelium (RPE), a monolayer of pigmented cells lining the photoreceptors cells, plays an important role in ARM pathophysiology.[137, 230] Endogenous or exogenous influences on the RPE may interfere with the homeostatic function of this metabolically highly active tissue and initiate or exacerbate pathological processes. An example of the exogenous influences is medication use, given the well-known fact that certain drugs, like chloroquine and chlorpromazine, are toxic to the RPE.[57, 137] Furthermore, the study of medication use as a potential risk factor for ARM may give relevant clues to a correlation between ARM and the disease for which the medication was prescribed. Only a few studies have included medication use in their risk analyses and they have produced mixed results.[7, 73, 82, 140] Klein et al. published a separate paper on this issue, but did not observe striking associations with the five-year incidence of early ARM.[123]

Investigations into the association between medication use and late ARM are hampered by the relative infrequency of this end-stage disease in the population. Moreover, since specific types of many drugs are not commonly used, the statistical power to demonstrate the presence or absence of a relationship is small, especially when adjusting for relevant confounders. Therefore, we decided to pool data on the incidence of early ARM, a recognized precursor of late ARM,[116, 145, 201] from three cohort studies that have previously cooperated in a risk factor analysis of prevalence data.[184] These population-based studies used similar methods regarding ARM grading and definition, and the assessment of medication use. Within this large dataset, we studied the association between use of different categories of medications and the incidence of early ARM.

MATERIAL AND METHODS

Study population

The study population consisted of participants in three population-based, prospective cohort studies: the Beaver Dam Eye Study (BDES), the Rotterdam Study (RS), and the Blue Mountains Eye Study (BMES). The BDES started in 1988 in Beaver Dam, Wisconsin, and included 4926 subjects (response 83.1%) aged 43-86 years, identified in a private census. The RS started in 1990 in a suburb of Rotterdam, the Netherlands, and included 7983 subjects (response 77.7%) aged 55-106 years, identified from the municipal registers. Because the ophthalmologic part of the RS became operational after the screening of participants had started, only 6780 (84.9%) participated in the ophthalmic examination. The BMES started in 1992 in the Blue Mountains area, west of Sydney, Australia, and included 3654 subjects (response 82.4%) aged 49-97 years, also identified in a private census. The three populations were almost entirely of northern European origin. All three studies were conducted in accordance with the tenets of the Declaration of Helsinki, and a written informed consent was obtained from all participants. Detailed information on

the identification and description of each study population has appeared in previous reports.[108, 142, 209]

Follow-up was conducted after a mean interval of 4.8 years in the BDES (range 2.6-7.1), 6.5 years in the RS (range 5.1-9.7), and 5.1 years in the BMES (range 3.0-7.8). Of the 4541 surviving participants in the BDES, 3684 (81.1%) subjects participated in the follow-up examination. In the RS, of the 5110 surviving participants, 3636 (71.2%) subjects participated at follow-up. The BMES follow-up examination included 2335 (75.1%) of the 3111 survivors. In the present study, only participants without early and late ARM (definitions are presented below) at baseline were included. The numbers for this sample were 3012 in the BDES, 3434 in the RS, and 2203 in the BMES, making a total of 8649 subjects at risk of early ARM in the pooled cohort. Comparisons between participants and non-participants at follow-up have been presented elsewhere.[116, 145, 201]

Procedures and definitions

All three studies employed similar diagnostic procedures and definitions for early and late ARM, which were the same at baseline and follow-up. From both eyes, stereo colour photographs were taken of the macular area. In the BDES and BMES, a Zeiss fundus camera was used and in the RS a Topcon TRV-50VT fundus camera. Grading of the fundus transparencies for the BDES and the BMES was based on the Wisconsin Age-Related Maculopathy Grading System.[107] In the RS grading was performed according to the International Classification System,[24] which is a modification of the Wisconsin System. Fundus transparencies were overlaid by a standardized grid and graded using a stereo-viewer. Follow-up photographs were graded in comparison with the baseline transparencies.

In the present study, early ARM was defined as the presence of either intermediate soft distinct drusen (≥63μm) with RPE depigmentation or hyperpigmentation, or soft indistinct drusen (≥125μm), or reticular drusen. Incidence of early ARM was defined as absence of early and late ARM in either eye at baseline and presence of early ARM in at least one eye at follow-up. Persons with late ARM in either eye at follow-up were excluded.

Medication use

In all three studies, exposure to medication was assessed at baseline by means of a standardized interview. Participants were asked what medication, both prescription and over-the-counter, they used at the time of the baseline examination or in the preceding week. Furthermore, participants were asked to bring their medications to the examination centres to verify the names of medications they were taking.

In the present study, a selection was made of categories of drugs that were available to all three populations and that were commonly used, defined by 1% or greater usage. Based on this criterion, antipsychotics, barbiturates, antiemetics, chloroquine, quinine, and antihistamines were excluded.

		Beaver Dam Eye Study	Rotterdam Study	Blue Mountains Eye Study	Pooled cohort	*P*-value*
Date of interview		1988-1990	1990-1993	1992-1994	1988-1994	
Number		3012	3434	2203	8649	
Age (years)		58.7 ± 9.8	65.4 ± 6.8	64.0 ± 8.4	62.7 ± 8.9	<0.001
Age (%)	<60 y	54.9	22.0	32.5	36.1	
	60-64 y	14.9	28.0	20.7	21.5	
	65-69 y	14.3	23.4	20.6	19.5	
	≥70 y	16.0	26.6	26.2	22.8	
Gender (% female)		55.8	57.7	57.1	56.9	0.92
Body mass index (kg/m^2)		28.8 ± 5.2	26.3 ± 3.5	26.3 ± 4.3	27.2 ± 4.5	<0.001
Systolic blood pressure (mmHg)		129.7 ± 18.9	135.6 ± 20.6	145.1 ± 20.4	136.0 ± 20.8	<0.001
Diastolic blood pressure (mmHg)		78.2 ± 10.4	73.6 ± 10.7	83.5 ± 9.8	77.8 ± 11.1	<0.001
Hypertension (%)		32.8	29.4	43.4	34.2	0.001
Total cholesterol (mg/dl)		232.7 ± 43.4	259.3 ± 45.7	233.7 ± 40.7	243.6 ± 45.5	<0.001
HDL-cholesterol (mg/dl)		52.4 ± 17.5	52.4 ± 13.7	55.4 ± 16.8	53.1 ± 15.9	<0.001
Diabetes mellitus (%)		8.2	6.9	4.8	6.8	<0.001
History myocardial infarction (%)		4.5	10.1	8.2	7.6	0.001
History of stroke (%)		2.0	2.2	3.1	2.4	0.50
Age at menopause (years)		45.7 ± 7.3	48.9 ± 5.0	46.9 ± 6.7	47.4 ± 6.4	<0.001
Smoking (%)	Never	44.5	33.0	51.5	41.7	
	Former	35.8	45.6	35.6	39.6	0.008
	Current	19.7	21.4	12.9	18.7	

*Linear or logistic regression analysis for differences between the three study populations adjusted for age and gender

Table 3.6.1 *Baseline characteristics of participants included in the analysis, for each study site separately and for the pooled cohort.*

Antihypertensive drugs included angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers, and beta-blockers (excluding topical medications). Thiazide, potassium-sparing and loop diuretics were included in the category of diuretics. Several subtypes of lipid-lowering drugs, including statins, were pooled because the statistical power for analyses of each subtype separately was low. Hormone replacement therapy (HRT) was defined as the use of oral estrogen and/or progesterone. Because the majority of sedatives consisted of benzodiazepines, only this category was included. For the same

reason, we only included tricyclic antidepressants. Of the topical medications, timolol eye drops were included as the most commonly used beta-blocker.

Confounders

The following variables available from all three studies were considered potential confounders in the association between medication use and early ARM: age, gender, body mass index, systolic blood pressure, diastolic blood pressure, smoking, serum total cholesterol, and high-density lipoprotein (HDL) cholesterol. Body mass index was calculated as weight divided by height squared (kg/m^2), and blood pressure was expressed per 10 mmHg. Smoking was assessed by interview and categorized as current, former, or never. Serum total cholesterol and HDL cholesterol were measured from non-fasting blood and expressed as mg/l.

Statistical analysis

For the present study, datasets from each study population were merged into one combined set. To study differences in baseline characteristics between populations, linear regression analysis with a study-site variable, adjusted for age and gender, was used for continuous variables and logistic regression analysis for dichotomous variables, including the use of medication. The association between current use of a specific category of medicine and the incidence of early ARM was analysed using logistic regression. Analyses were performed in the pooled dataset and in each study population separately. Each model was adjusted for age, gender, and time of follow-up. Also, to adjust for residual differences among the three study populations, a categorical variable representing data source was included in all analyses with the pooled dataset. In a subsequent model in the pooled cohort only, additional adjustment was made for other potential confounders, as mentioned previously. To assess heterogeneity, i.e. whether the effect of medication use is unequal across the study populations, an interaction term of data source and medication of interest was constructed and tested with the Wald test. All statistical analyses were performed using SPSS for Windows version 11 (SPSS Inc., Chicago, Illinois).

RESULTS

Baseline characteristics of the separate studies and of the pooled cohort are presented in Table 1. In short, the pooled population totalled 8649 participants aged 43 to 93 years (mean 62.7 years). The mean age of participants in the BDES (58.7 years) was lower than in the RS (65.4 years) and BMES (64.0 years; $P<.001$). The pooled cohort included 4921 women and 3728 men. Except for sex distribution and a history of stroke, all baseline characteristics differed significantly between the three study populations (Table 1). The total number of subjects with incident early ARM in the pooled cohort was 683 (7.9%). The incidence of early ARM, adjusted for age, gender, and follow-up time, was significantly lower in the RS compared to the BDES and BMES. The

	Beaver Dam Eye Study		Rotterdam Study		Blue Mountains Eye Study		Pooled Cohort	
	No.	%	No.	%	No.	%	No.	%
All antihypertensives	431	14.3	648	18.8	593	26.9	1671	19.3
ACE inhibitors	169	5.8	159	4.6	148	6.7	476	5.6
Calcium channel blocker	56	2.0	156	4.5	237	10.8	449	5.3
Beta-blockers	230	8.2	437	12.7	303	13.8	970	11.5
Cardiac glycosides	74	2.5	51	1.5	65	3.0	190	2.2
All diuretics	640	21.2	300	8.7	278	12.6	1218	14.1
Thiazide	537	20.0	228	6.6	166	7.5	931	11.2
K-sparing diuretics	235	8.7	21	0.6	141	6.4	397	4.8
Loop diuretics	69	2.6	66	1.9	96	4.4	231	2.8
All lipid lowering agents	88	3.0	93	2.7	79	3.6	260	3.0
All NSAIDs	972	32.3	224	6.5	316	14.3	1512	17.5
Acetaminophen	263	8.7	750	21.8	103	4.7	1116	12.9
Steroids	51	1.7	43	1.3	34	1.5	128	1.5
HRT*	214	12.7	55	2.8	212	18.5	481	10.0
Thyroid stimulating agents	143	5.0	56	1.6	88	4.0	287	3.4
Benzodiazepines	244	8.6	370	10.8	136	6.2	750	8.9
Tricyclic antidepressant	78	2.8	23	0.7	39	1.8	140	1.7
Timolol eye drops	34	1.1	30	0.9	38	1.7	102	1.2

* Percentage based on women only
ACE = angiotensin converting enzyme
K-sparing = potassium-sparing
NSAIDs = non-steroidal anti-inflammatory drugs
HRT = hormone replacement therapy

Table 3.6.2 *Number and percentage of participants using a specific type of medication during the week of the baseline interview.*

odds ratios (OR) of early ARM in the BDES and BMES relative to the RS were 1.5 (95% confidence interval (CI) 1.1, 2.2) and 1.3 (95% CI 1.0, 1.8), respectively. When we also adjusted for smoking, these risk estimates increased to 1.6 and 1.4, respectively.

Of all subjects in the pooled cohort, 4606 (53.3%) used at least one of the medications selected for this study. For each category of medication, the number of participants using the drug at baseline is presented in Table 2.

Statistically significant differences ($P<.01$) between study populations, adjusted for age and gender, existed for all categories except for lipid lowering drugs and steroids. In the BDES, less calcium channel blockers and beta-blockers were used compared with the RS and BMES, while more diuretic use was reported in the US population. Compared with the BDES and BMES, few women in the RS used HRT.

In the pooled cohort, users of antihypertensive medication had a borderline statistically significant increased risk of incident early ARM (OR 1.2; 95% CI 1.0, 1.4) while adjusting for age, gender, data source, and time of follow-up (Table 3, model 1). This association was mainly accounted for by beta-blockers, which as a separate category showed an OR of 1.2 (95% CI 1.0, 1.5). Additional adjustment for body mass index, systolic and diastolic blood pressure, smoking, total and HDL cholesterol (model 2), strengthened the relation (OR 1.3; 95% CI 1.0, 1.6). Approximately the same OR's were observed in the three studies separately, although these risk estimates did not reach statistical significance. Adjusted for all confounders, a protective effect of borderline significance was found for the use of HRT (OR 0.6, 95% CI 0.4, 1.0) in the pooled data. The same effect was seen in the BDES and BMES separately but not in the RS. In the BMES, a borderline significant relationship was found between benzodiazepines and the incidence of early ARM (OR 1.7; 95% CI 1.0, 2.9), but this association was not present for either of the other two sites or in the pooled cohort. Also of borderline statistical significance was an association in the pooled cohort between the use of tricyclic antidepressants and incident early ARM (OR 0.4; 95% CI 0.2, 1.0), adjusted for all confounders. Topical beta-blockers were not associated with the incidence of early ARM (OR 1.2; 95% CI 0.6, 2.1). We also found no association between incident early ARM and other categories of drugs, in particular ACE inhibitors (OR 1.1; 95% CI 0.8, 1.5), calcium channel blockers (OR 1.2; 95% CI 0.8, 1.6), and non-steroidal anti-inflammatory drugs (NSAIDs; OR 0.9; 95% CI 0.8, 1.2). For all analyses the test for heterogeneity was negative.

DISCUSSION

In this large pooled cohort we found borderline statistically significant evidence for an increased risk of early ARM among users of antihypertensive medication and a decreased risk among women taking HRT and persons using tricyclic antidepressants. The observed associations were of borderline statistical significance and were not altered when adjusted for appropriate confounders.

Before we may interpret these findings, some methodological issues need to be discussed. Inevitably, there were differences among the three study populations in participation rates, definitions of confounders, and indications for medication use. Also, the incidence of early ARM differed across the studies, possibly due to differences in grading. To correct for these differences in the pooled cohort, we included in all regression models a variable representing data source. Moreover, we tested for heterogeneity, i.e. whether

	Beaver Dam Eye Study		Rotterdam Study		Blue Mountains Eye Study		Pooled cohort			
							Model 1		Model 2	
	OR	(95%CI)	OR	(95%CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
All antihypertensives	1.1	(0.8, 1.7)	1.1	(0.8, 1.5)	1.2	(0.9, 1.7)	1.2	(1.0, 1.4)	1.2	(1.0, 1.5)
ACE inhibitors	0.8	(0.4, 1.6)	1.2	(0.7, 2.1)	1.0	(0.6, 1.7)	1.0	(0.7, 1.4)	1.1	(0.8, 1.5)
Calcium channel blockers	1.6	(0.7, 3.6)	0.7	(0.4, 1.3)	1.3	(0.8, 2.0)	1.1	(0.8, 1.5)	1.2	(0.8, 1.6)
Beta-blockers	1.2	(0.7, 1.9)	1.2	(0.8, 1.7)	1.3	(0.9, 2.0)	1.2	(1.0, 1.5)	1.3	(1.0, 1.6)
Cardiac glycosides	0.6	(0.3, 1.5)	0.5	(0.2, 1.7)	0.7	(0.3, 1.6)	0.6	(0.4, 1.1)	0.6	(0.4, 1.1)
All diuretics	0.9	(0.7, 1.3)	1.1	(0.8, 1.7)	1.2	(0.8, 1.8)	1.1	(0.9, 1.3)	1.1	(0.9, 1.4)
Thiazide	0.9	(0.7, 1.3)	1.1	(0.7, 1.7)	1.1	(0.6, 1.8)	1.0	(0.8, 1.3)	1.0	(0.8, 1.3)
K-sparing diuretics	1.0	(0.6, 1.6)	1.3	(0.4, 4.7)	1.3	(0.8, 2.3)	1.2	(0.8, 1.6)	1.2	(0.9, 1.7)
Loop diuretics	1.4	(0.7, 2.9)	1.0	(0.5, 2.3)	1.5	(0.8, 2.6)	1.4	(0.9, 2.0)	1.4	(0.9, 2.0)
All lipid lowering agents	1.0	(0.5, 2.3)	1.6	(0.8, 3.2)	0.4	(0.1, 1.1)	0.9	(0.6, 1.5)	1.0	(0.6, 1.6)
All NSAIDs	0.9	(0.7, 1.2)	0.8	(0.5, 1.4)	1.1	(0.8, 1.7)	0.9	(0.8, 1.2)	0.9	(0.8, 1.2)
Acetaminophen	1.1	(0.6, 1.7)	0.9	(0.7, 1.3)	1.5	(0.8, 2.8)	1.0	(0.8, 1.3)	1.0	(0.8, 1.3)
Steroids	1.3	(0.5, 3.3)	1.8	(0.7, 4.4)	0.6	(0.1, 2.7)	1.3	(0.7, 2.3)	1.3	(0.7, 2.4)
HRT*	0.6	(0.3, 1.2)	1.1	(0.3, 3.6)	0.6	(0.3, 1.2)	0.6	(0.4, 1.0)	0.6	(0.4, 1.0)
Thyroid stimulating agents	1.3	(0.7, 2.3)	1.6	(0.7, 3.7)	0.8	(0.4, 1.8)	1.2	(0.8, 1.8)	1.2	(0.8, 1.8)
Benzodiazepines	0.6	(0.4, 1.1)	1.0	(0.7, 1.5)	1.7	(1.0, 2.9)	1.0	(0.8, 1.3)	1.0	(0.8, 1.3)
Tricyclic antidepressants	0.3	(0.1, 1.1)	0.5	(0.1, 3.7)	1.1	(0.4, 2.9)	0.6	(0.3, 1.2)	0.4	(0.2, 1.0)
Timolol eye drops	1.5	(0.6, 3.6)	0.6	(0.1, 2.7)	1.0	(0.3, 2.9)	1.1	(0.6, 2.0)	1.2	(0.6, 2.1)

* Association based on women only ACE = angiotensin converting enzyme K-sparing = potassium-sparing
NSAIDs = non-steroidal anti-inflammatory drugs HRT = hormone replacement therapy

Table 3.6.3 *Associations (Odds Ratios) between medication use and the incidence of early ARM. All associations were adjusted for age, gender, follow-up time, and data source for the pooled cohort (model 1). Additional adjustment was made for systolic blood pressure, diastolic blood pressure, total and HDL cholesterol, body mass index, and smoking (model 2). Current use of medication was compared with past or never use.*

the medication effect is different across the study populations. Because such a difference is biologically not plausible, we would have considered the presence of heterogeneity as an argument against a true association between ARM and medication use.

No baseline data were available on the dose and duration of medication usage from all three studies. This may have resulted in misclassification of exposure by including both one-time users and high-dose, long-term consumers. Since this is non-differential misclassification, which occurs to the same extent in both the cases and the controls, we may have underestimated the true effect.

As in all pharmaco-epidemiological studies, confounding-by-indication is an important potential problem. Confounding-by-indication denotes the phenomenon that the relation found between medication use and a disease is representative of an association between a risk factor for which the medication was prescribed and the disease. With antihypertensive drugs this may be the case since hypertension has been implicated as a risk factor of ARM.[127, 186] Though we adjusted for both systolic and diastolic blood pressure separately, residual confounding cannot be excluded. Therefore, we are not able to discriminate between a possible direct toxic effect of beta-blockers and an effect of high blood pressure in itself. If the latter is true, our results support the idea that high blood pressure increases the risk of ARM. Our finding that topical beta-blockers were not associated with incident early ARM suggests that a toxic effect from beta-blocker use is unlikely, provided that topical beta-blockers reach the retina. Finally, because we performed multiple analyses with a wide range of medicines, our findings could be due to chance alone.

In the epidemiological literature, few data are available on the relation between medication use and ARM. Hirvelä et al. found no association between prevalent ARM and antihypertensive medication, aspirin or sedatives.[73] Hyman et al. for the Age-Related Macular Degeneration Risk Factor Study Group demonstrated a relation between neovascular AMD and the use of antihypertensive medication.[82] However, this effect could not be separated from an independent effect of hypertension. In the Age-Related Eye Disease Study, persons with large drusen were more likely to use hydrochlorothiazide diuretics and those with geographic atrophy were more likely to use antacids and thyroid hormones.[7] Klein et al. studied a range of medications in relation to the incidence of early ARM.[123] They found a few borderline statistically significant associations; however, a significantly lower incidence was found in subjects using any type of antidepressant. Past or current use of ACE inhibitors was associated with both ARM and AMD in the Visual Impairment Project (VIP).[140] Since data on blood pressure were not available from the VIP study, appropriate adjustment for this confounder could not be performed. A recent BMES report showed no association between NSAIDs or corticosteroids and the prevalence or incidence of ARM.[213]

Interestingly, the observed effect of beta-blockers in our study is in line with an in vitro study by Ellis et al. [Invest Ophthalmol Vis Sci 40 (Suppl):224,

1999] that investigated whether exposure to specific antihypertensive drugs affected lipofuscin deposition within cultured human RPE cells. They observed that three drug regimens, the beta-blocker propranolol and the calcium antagonists verapamil and diltiazem, induced cellular fluorescence indicating the presence of lipofuscin-like material. The exact mechanism and meaning of this finding is not known, but deserves further study.

Special interest has been paid to the association between HRT and ARM. The Eye Disease Case Control Study was the first to note a protective effect of postmenopausal estrogens.[3] In 1995, Vingerling et al. found in the Rotterdam Study that women with early artificial menopause carried an increased risk of late ARM, also suggesting that estrogens may play a protective role in the pathogenesis of this disease.[211] Later studies, however, have produced inconsistent results.[101, 103, 180] In the present study, a non-significant protective effect of HRT was found in the BDES and BMES, whereas this effect was of borderline significance in the pooled cohort. The non-significant increased risk found in the RS may have been due to the low number of women taking HRT (n=59) at baseline in this population.

Another relationship that has attracted much attention recently is that between cholesterol-lowering medication and ARM. Some authors have suggested that this type of medication may reduce the risk of ARM,[67, 141] but others have refuted this.[105, 202] The results from this study do not support the hypothesis that cholesterol-lowering medication protect against the development of early ARM.

The inverse association of antidepressants with ARM in the BDES was reported previously.[123] In the present study that included the BDES population, the relationship was confirmed in the RS and the pooled cohort, but not in the BMES. We are not aware of a biological link that may explain the observed protective effect. Tricyclic antidepressants are tertiary amines that block the uptake of noradrenaline and serotonin by brain synaptosomes, but their effect on the retina is not known.

To conclude, in this large pooled dataset based on three population-based studies, we did not find strong relationships between baseline medication use and the incidence of early ARM. A slightly increased risk was found for antihypertensive medication, in particular beta-blockers. This association was consistent among the three study populations and did not alter after adjustment for appropriate confounders, including blood pressure. Possible protective effects were observed for HRT and tricyclic antidepressants. The observed associations could reflect the effect of unmeasured or uncontrolled confounders, e.g. diet or hypertension, or simply chance findings. Still, our findings warrant further investigations into the effect of these medications on the development of ARM, preferably including information on dosage and duration of drug exposure.

3.7 Cholesterol lowering drugs and risk of age related maculopathy: prospective cohort study with cumulative exposure measurement

We tested the hypothesis that cholesterol-lowering drugs protect against age-related maculopathy in a large cohort study with cumulative exposure measurement. In contrast to previous claims did we not observe any effect of these drugs on the risk of age-related maculopathy, making a protective effect of statins unlikely. ***BMJ 2003;326:255-256.***

Recently, two studies have claimed that cholesterol lowering drugs, in particular statins, protect against age related maculopathy (ARM).[67, 141] The end stage of this progressive retinal disorder is the commonest cause of incurable blindness in elderly people in Western societies, and its prevalence is expected to rise with the ageing of the population. Thus, preventing this disorder would have an enormous public health impact.[16] The above-mentioned studies used interview data on drug use and had a low statistical power. We therefore tested the hypothesis that cholesterol-lowering drugs protect against ARM in a large cohort study with cumulative exposure measurement.

Participants, methods, and results

This investigation was part of the Rotterdam Study, a population based prospective cohort study of people aged 55 years and more. After the baseline phase from 1990 to 1993, two follow-up examinations were performed at mean intervals of 2 and 6.5 years. Of all the subjects at risk of ARM, 4822 (83%) participated at follow-up. A diagnosis of ARM was based on stereoscopic colour fundus transparencies graded according to the international classification system.[24] The incidence of the disorder was defined as the

	No. of subjects with incident ARM (n = 419)	No. of subjects in total cohort (n = 4822)	Crude hazard ratio* (95% confidence interval)	Adjusted hazard ratio† (95% confidence interval)
All drugs				
No exposure‡	391	4365	1.0	1.0
<1 month exposure	2	26	1.1 (0.3, 4.3)	1.2 (0.3, 5.0)
1-<12 months exposure	8	136	1.0 (0.5, 2.1)	1.0 (0.5, 2.0)
≥12 months exposure	18	295	1.0 (0.6, 1.6)	1.2 (0.7, 1.9)
Statins				
No exposure¶	394	4407	1.0	1.0
<1 month exposure	2	21	1.5 (0.4, 6.1)	1.6 (0.4, 6.5)
1-<12 months exposure	7	120	1.0 (0.5, 2.1)	0.9 (0.4, 2.1)
≥12 months exposure	16	274	1.0 (0.6, 1.6)	1.1 (0.7, 1.9)

*Adjusted for age and gender
†Additional adjustment for body mass index, smoking, hypertension and peripheral arterial disease
‡In this time dependent analysis, cumulative drug exposure of each case was compared with that of all other subjects in the cohort as controls, on the index date half way between the two examinations when the incident case occurred. Controls may contribute more than once. Hence, relative risks cannot be calculated with the numbers given in the table.

Table 3.7 *Hazard ratios of age related maculopathy (ARM) associated with the use of cholesterol lowering drugs.*

development of soft distinct drusen with pigmentary irregularities, indistinct drusen, or the end stages of atrophic or neovascular age related macular degeneration.

A register of prescriptions filled by local pharmacies provided continuous data on use of cholesterol lowering drugs. These data were available for 99% of the cohort from 1 January 1991 onwards. We used Cox proportional hazards regression analysis to calculate hazard ratios, with age in days as the time axis to ensure optimal controlling for age. Cumulative exposure to drugs was represented as a time dependent covariate and was analysed both as a dichotomous and a categorical variable. The model compared each incident case of ARM with all subjects in the cohort who were alive and free of the disorder at the age when the case of ARM was diagnosed.[85]

During 26,781 person years of follow-up, 457 subjects used cholesterol-lowering drugs for one or more days, and 419 cases of incident ARM were observed. Use of cholesterol lowering drugs at any time, defined as a binary variable, was not associated with the incidence of ARM (hazard ratio 1.0; 95% confidence interval 0.7, 1.5). Compared with persons who had never used cholesterol-lowering drugs, cumulative exposure for less than one month, for one month to a year, or for more than a year did not have a protective effect on the risk of ARM (see Table 3.7). Additional adjustment for body mass index, hypertension, smoking, and peripheral arterial disease (ankle-arm index < 0.9) did not change the association. When we performed the same analysis with progression of ARM as outcome variable, we obtained the same results.

Comment

Exposure to cholesterol lowering drugs did not change the risk of ARM. In contrast with the studies that reported a protective effect, we used a prospective design and assessed drug use by means of data registered by pharmacies. This minimised potential selection and information bias, and our data provided quantitative information for each participant's cumulative exposure to drugs. This prevented misclassification of the duration of drug use. Even though the total number of participants was high, the number of subjects using cholesterol-lowering drugs who developed ARM was low, possibly leading to a type II error. With a two sided α of 0.05, we had a power of 80% to show a relative risk of 0.7 or lower. The fact that we did not find an association between cholesterol lowering drugs and ARM makes a protective effect of statins unlikely.

4
Prognosis

Is there a direct association between age-related eye diseases and mortality?

Purpose: *To study mortality in subjects with age-related maculopathy (ARM), cataract, or open-angle glaucoma (OAG) in comparison with persons without these disorders.* ***Design:*** *Population-based prospective cohort study.* ***Participants:*** *Subjects (n=6339) aged 55 years or older from the population-based Rotterdam Study for whom complete information on eye disease status was present.* ***Main outcome measures****: Vital status, continuously monitored from March 1990 until January 2000.* ***Methods:*** *The diagnosis of ARM was made according to the International Classification System. Cataract, determined on biomicroscopy, was defined as any sign of nuclear or (sub)cortical cataract, or both, in at least one eye with a visual acuity of 0.5 or less. Aphakia and pseudophakia in at least one eye were classified as operated cataract. Definite OAG was defined as a glaucomatous optic neuropathy combined with a glaucomatous visual field defect. Diagnoses were assessed at baseline. Mortality hazard ratios were computed using Cox proportional hazard regression analysis, adjusted for appropriate confounders (age, gender, smoking status, body mass index, cholesterol level, atherosclerosis, hypertension, history of cardiovascular disease, and diabetes mellitus).* ***Results:*** *The adjusted mortality hazard ratio for subjects with AMD (n=104) was 0.94 (95% confidence interval [CI] 0.52-1.68), with biomicroscopic cataract (n=951) was 0.94 (95% CI 0.74-1.21), with surgical cataract (n=298) was 1.20 (95% CI 0.86-1.68), and with definite OAG (n=44) was 0.39 (95% CI 0.10-1.55).* ***Conclusion:*** *Both ARM and cataract are predictors of shorter survival because they have risk factors that also affect mortality. When adjusted for these factors, ARM, cataract, and OAG were themselves not significantly associated with mortality.* ***Ophthalmology 2003;110:1292-1296.***

Previous studies on the relation between mortality and age-related maculopathy (ARM), cataract, and open-angle glaucoma (OAG) have suggested a higher mortality in affected subjects.[53, 106, 196] However, for patients with end-stage ARM, also known as age-related macular degeneration (AMD), and for patients with OAG, this association disappeared in most studies after adjustment for age, gender and survival-related factors (e.g. diabetes mellitus).[21, 113, 130] Cataract, in general, was thought to have no relationship with survival,[130] but more recently nuclear cataract has been reported to be an independent indicator of mortality.[113, 223] It may be that mortality is directly related to these eye diseases, for example because of

higher accident rates or complications of therapies such as surgery, eye drops, or other medications. To study whether there is an association, and if so, if it is the result of shared risk factors, we examined a large cohort in which a range of disorders was studied over a long period of follow-up.

METHODS

Study population

The present study was performed within the Rotterdam Study, a prospective cohort study of all residents aged 55 years and older of a suburb of Rotterdam.[74] The Declaration of Helsinki was followed. All persons living in this suburb were asked to participate in an extensive standardized home interview. After written informed consent was obtained, they received an appointment for a medical examination, including an ophthalmologic one, between 1990 and 1993. Of 6780 subjects who underwent a ophthalmologic examination, 6,339 subjects (92.2%) had sufficient data to establish a diagnosis of ARM, cataract, or OAG.

Ophthalmologic examination

Three ophthalmologic residents and two technicians performed the ophthalmologic examination. The exact procedure has been described previously.[227] In short, this examination included keratometry, autorefraction followed by subjective adjustment, slit lamp biomicroscopy, tonometry, ophthalmoscopy, and perimetry. Simultaneous stereo colour transparencies of the optic disc and mono and stereo colour transparencies of the macula were obtained.

Diagnosis of eye disease

The diagnosis of ARM was based on the grading of colour fundus transparencies according to the International Classification System, in which all fundus signs of age related maculopathy are called ARM.[24] Its two end stages (atrophic and neovascular macular degeneration) are called AMD or late ARM, and the earlier stages are also known as early ARM.

The diagnosis of cataract was based on biomicroscopy evaluation. Operated cataract in a subject was present when at least one eye had undergone a lens extraction (with or without artificial lens implantation). Biomicroscopic cataract was classified as being present when any sign of nuclear or (sub)cortical cataract was observed in at least one eye with a visual acuity of 0.5 or less and no visible fundus change explaining this loss.The diagnosis of definite OAG was based on the combination of a possible glaucomatous optic neuropathy (GON) and a glaucomatous visual field defect (GVFD).[227] Probable OAG cases had either a probable GON without a GVFD or a GVFD without possible GON. Possible OAG cases were those subjects who had a possible GON without a GVFD. Since pseudo-exfoliation as a cause of OAG was not excluded at baseline, we use here the term OAG instead of POAG.

Other baseline measurements

Age, gender, smoking status, body mass index, cholesterol level, atherosclerosis, hypertension, history of cardiovascular disease, and diabetes mellitus were considered possible confounding indicators of mortality. Smoking was analysed as a categorical variable and classified into never, former or current smoker. Body mass index was calculated as weight divided by height^2. Serum cholesterol was determined with an automatic enzymatic procedure (Boehringer Mannheim Systems, Mannheim, Germany). As measures of atherosclerosis, the ankle-arm index, intima-media thickness of the common carotid artery, and presence of calcifications in the abdominal aorta were used. The ankle-arm index was calculated by dividing the lower systolic blood pressure of the posterior tibial artery of the two legs by the average systolic blood pressure at the right arm. An ankle-arm index below 0.9 was indicative of atherosclerosis. Intima-media thickness was measured on ultrasonographic images of both the left and right carotid artery.[28] The presence of calcifications in the abdominal aorta was determined using a lateral radiograph of the lumbar spine (L1-L4).[226] Criteria for hypertension were a systolic blood pressure of 160 mm Hg or diastolic blood pressure of 95 mm Hg or more or the use of antihypertensive medication for the indication of hypertension. Subjects were considered to have a history of cardiovascular disease when a myocardial infarction or stroke was reported and subsequently confirmed by electrocardiogram or by reviewing the medical records from the general practitioner or medical specialist. Diabetes mellitus was defined as the use of blood glucose-lowering medication, a fasting serum glucose level of 11.1 mmol/l or more, or both.[6]

Mortality

From March 1990 until January 1, 2000, the vital status of the participants was obtained from the municipal registry 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, 2000, whichever came first. Data on vital status was nearly complete (99.9%).

Statistical analysis

Analysis of co-variance adjusting for age and gender was used to compare baseline characteristics between diseased and non-diseased subjects. To study differences in survival, Cox proportional hazard regression analysis was used with age at baseline on the time axis (SAS Statistical software; SAS Institute Inc. Cary, NC, USA). All potential confounders were initially included in the model. To assess which variables should be included as confounders, a Cox proportional hazard regression analysis was performed (results not shown in the text) with the backward stepwise method.

	No ARM† (n=5758)	AMD† (n=104)	No cataract (n=5090)	Biomicroscopic cataract (n=951)	Operated cataract (n=298)	No OAG† (n=5155)	Definite OAG† (n=44)
Mean (standard deviation)							
Age, years	68.2 (8.4)	82.0 (8.2)**	66.8 (7.5)	77.6 (7.5)**	77.2 (8.2)**	68.7 (8.7)	72.3 (6.5)**
Ankle-arm index	1.07 (0.22)	0.91 (0.26)**	1.09 (0.20)	0.96 (0.28)**	0.95 (0.28)**	1.07 (0.23)	1.10 (0.26)*
Body mass index, kg/m^2	26.3 (3.70)	26.0 (3.8)	26.3 (3.6)	26.4 (4.13)	26.1 (3.6)	26.3 (3.7)	25.9 (3.4)
Cholesterol, mmol/l	6.65 (1.21)	6.26 (1.19)	6.66 (1.20)	6.50 (1.24)	6.47 (1.27)	6.65 (1.21)	6.58 (1.22)
Intima-media thickness, μm	0.79 (0.16)	0.90 (0.15)	0.78 (0.15)	0.86 (0.16)	0.88 (0.18)**	0.80 (0.16)	0.80 (0.16)
Percentage							
Women	59.2	65.4	57.7	67.1**	48.6	59.6	47.7*
Primary education only	25.6	31.2	26.0	22.4	26.0	26.4	23.9
Smokers, ever	65.7	60.0**	68.0	52.8	58.6	65.5	59.1
Cardiovascular disease history	3.0	12.8**	2.4	6.6**	9.2**	3.2	2.9
Diabetes mellitus	10.3	18.5	8.94	16.6**	16.3	10.1	14.0
Hypertension	34.0	38.0**	33.0	43.0*	47.0	35.0	43.0

ARM = age-related maculopathy; AMD = age-related macular degeneration; OAG = open angle glaucoma
†Early ARM and possible and probable OAG were left out
*Statistically significant difference at 0.10 level, corrected for age and gender
**Statistically significant difference at 0.05 level, corrected for age and gender

Table 4.1 *Baseline characteristics of the study population (n=6339) for subjects with and without age-related maculopathy, cataract and open-angle glaucoma.*

	Total	Deceased (%)		Total survival time
Age-related maculopathy (ARM)				
No ARM	5758	1120	(19.5)	40,913
Early ARM	477	174	(36.5)	3099
Late ARM	104	65	(62.5)	524
Total	6339	1359	(21.4)	44,536
Cataract				
No cataract	5090	808	(15.9)	36,953
Biomicroscopic cataract	951	405	(42.6)	5835
Operated cataract	298	146	(50.0)	1748
Total	6339	1359	(21.4)	44,536
Open-angle glaucoma (OAG)				
No OAG	5155	1081	(20.9)	36,309
Possible OAG	980	219	(22.3)	6817
Probable OAG	160	47	(29.4)	1086
Definite OAG	44	12	(27.3)	324
Total	6339	1359	(21.4)	44,536

Table 4.2 *Number (percentage) of deceased subjects and survival time (in person-years) according to disease status at baseline*

RESULTS

Table 4.1 shows the baseline characteristics of the subjects according to their eye disease status. All diseased subjects were older than the non-diseased ones. Subjects with cataract and AMD had a lower ankle-arm index while subjects with definite OAG had a higher one than that of controls. Subjects with AMD and cataract more often had a history of cardiovascular disease. Subjects with AMD were less likely to be smokers than control persons, but after correction for age and gender, there was a positive significant relationship between smoking and AMD. During a total of 44,536 person-years of follow-up, 1359 subjects died. Mean follow up was 7.0 years (standard deviation, 1.95).

Table 4.2 shows the number of deceased subjects per eye disease. Of the 104 subjects with AMD, one had definite OAG, 81 had biomicroscopic cataract, 15 were operated for cataract, and seven had AMD only. There was no relationship between AMD and the type of cataract (data not shown). Of the 44 subjects with OAG, 13 had biomicroscopic cataract and eight operated cataract.

	Crude hazard ratio (95% CI)	Adjusted hazard ratio* (95% CI)	Adjusted hazard ratio† (95% CI)
Age-related maculopathy (ARM)			
Early ARM	2.08 (1.77-2.44)	1.08 (0.92-1.27)	0.96 (0.71-1.29)
Late ARM	4.80 (3.74-6.16)	1.35 (1.04-1.75)	0.94 (0.52-1.68)
Cataract			
Biomicroscopic cataract	3.25 (2.89-3.66)	1.19 (1.04-1.36)	0.94 (0.74-1.21)
Operated cataract	3.94 (3.30-4.70)	1.45 (1.20-1.75)	1.20 (0.86-1.68)
Open-angle glaucoma (OAG)			
Possible OAG	1.08 (0.94-1.25)	0.98 (0.85-1.14)	1.01 (0.78-1.28)
Probable OAG	1.45 (1.09-1.95)	0.97 (0.73-1.30)	0.81 (0.48-1.36)
Definite OAG	1.23 (0.70-2.17)	0.87 (0.49-1.54)	0.39 (0.10-1.55)

*Adjusted for age and gender
†Adjusted for age, gender, atherosclerosis, hypertension, diabetes mellitus, smoking, history of cardiovascular disease, body mass index and cholesterol level

Table 4.3 *Mortality hazard ratios for three age-related eye diseases.*

Table 4.3 shows the mortality hazard ratios for subjects with ARM compared with those without ARM. The unadjusted hazard ratios for both early and late ARM showed a strong association with mortality, which in the case of AMD was still present after correction for age and gender. However, after correction for additional confounders (smoking, body mass index, cholesterol level, atherosclerosis, hypertension, history of cardiovascular disease, and diabetes mellitus) the association disappeared. Also for cataract, both the unadjusted and the age- and gender-corrected hazard ratios showed a relationship with mortality, which disappeared after correction for additional confounders. The unadjusted hazard ratios for OAG showed a borderline significant association with survival, which disappeared after correction for age and gender.

DISCUSSION

The main finding in our study was that any association between age-related eye diseases (ARM, cataract, and OAG) and mortality could be explained by shared risk factors.

Some methodological issues have to be addressed prior to accepting this finding. The Rotterdam Study created a good opportunity to study mortality, in

the first place because we had long-term follow-up of survival for nearly all subjects. Furthermore, the study was performed in a population-based cohort instead of in a group of clinic-derived patients. Also, one of the most important indicators of mortality, atherosclerosis, was measured in three objective ways (ankle-arm index, carotid intima-media thickness and calcifications in the abdominal aorta).

Our results partially support literature data with regard to ARM. Neither the Beaver Dam Eye Study[113] nor the Blue Mountains Eye Study[215] found an association between ARM and mortality. The Beaver Dam Eye Study also did not find the crude associations that we did. A difference between the Beaver Dam Eye Study and the Rotterdam Study, which may explain this inconsistent finding, was that the Beaver Dam Eye Study analysed ARM as a continuous variable, while we put early ARM and AMD as categorical variables into the model. However, when performing the analysis in the same way as the Beaver Dam Eye Study did, we still found a statistically significant association when we corrected for age and gender only.

With regard to cataract, our results are also partially in agreement with the two most recent larger studies, the Beaver Dam Eye Study and the Salisbury Eye Evaluation Project.[113, 223] In concordance with our study, the Beaver Dam Eye Study found an association between cataract and mortality, which disappeared after correction for systemic variables. The Salisbury Eye Evaluation Project, however, reported a relationship between cataract and mortality even after correcting for age, race, gender, smoking, BMI and co-morbid conditions (self-reported stroke, cancer, parkinsonism, arthritis, myocardial infarction, congestive heart failure, and pulmonary problems). We found a relationship between cataract and mortality when we corrected for age and gender only, but this disappeared after correcting for all additional confounders. The difference could be explained by different confounder definitions. All data for co-morbid conditions in the Salisbury Eye Evaluation Project were self-reported. In our study the confounders were either measured in an objective way or verified when they were self-reported. The Beaver Dam Eye Study, the Blue Mountains Eye Study, and the Salisbury Eye Evaluation Project all reported an independent relationship between nuclear cataract and mortality (the Beaver Dam Eye Study only in people without diabetes). Unfortunately, we were not able to perform this subtype analysis because at baseline no distinction was made between cortical and nuclear cataract. Instead**,** all subtypes were grouped into one biomicroscopic cataract group. This prevented us from analysing our results for each subgroup separately, and therefore a possible effect in a subgroup, as described in previous studies, could not be detected.[113, 215, 223]

In our study, OAG was not related to mortality, which corresponds to findings from the literature.[113, 130] Previous studies on the association between OAG and mortality-related factors showed that there was no association between smoking and OAG.[99, 156] Results about an association between OAG and vascular factors or diabetes mellitus are inconclusive.[52, 51, 89, 100, 143, 197] Our

study shows that even if such an association were present, it would not be a strong one, because these factors do not have a strong confounding effect on the relationship between OAG and mortality.

The finding that ARM and cataract are predictors of survival contrary to OAG could be explained by the fact that ARM and cataract have risk factors that are more strongly related to mortality than the risk factors for OAG. It is thought that ARM is associated with cardiovascular disease and smoking,[119, 184, 208] and cataract with diabetes mellitus.[102, 131, 139, 222] These risk factors also are strongly related to mortality and therefore are powerful confounders in the relationship between ARM and cataract on the one hand and mortality on the other.

Based on our study, we can conclude that the age-related eye diseases ARM and cataract are predictors of survival because they share risk factors of mortality. When adjusted for these factors, neither ARM nor cataract was associated with mortality.

5
General discussion

The objectives of the studies described in this thesis were the assessment of the risk of age-related maculopathy (ARM) and the identification of its risk factors. For this purpose, I determined the incidence of ARM after six and a half years of follow-up in the Rotterdam Study, and investigated associations with endogenous and exogenous factors. In this final chapter, I will discuss several aspects of the epidemiological methodology in more depth, summarize the main findings, and integrate the results to come to an overall aetiological model. Moreover, I will consider the clinical relevance of the findings and provide suggestions for future epidemiological studies on the aetiology of ARM.

METHODOLOGY

Study design and population

Except for Chapters 2.2 and 3.6, all studies in this thesis were performed as part of the Rotterdam Study. This is an observational, prospective, population-based cohort study. The inclusion criteria for the study population were age 55 years and older and residence in Ommoord, a suburb of Rotterdam. Of the 10,750 eligible subjects, 7983 responded to the invitation. The advantages of such a large cohort study are numerous. The large size of the cohort allowed for reliable estimates of frequency and associations. The population-based sample greatly reduced the potential for selection bias. In contrast to a clinic-based study, the inclusion of persons was random and not distorted by determinants related to disease. Finally, the prospective design made it possible to determine a temporal relationship, which is the most important criterion for causality. Also, because the assessment of risk factors was performed prior to the onset of disease, information bias was limited. Because of the huge investments, both in terms of time and money, these large cohort studies are rare.

Population-based cohort studies on medication use as a determinant of disease require even larger numbers of participants. These studies lack statistical power, because of the infrequent use of most types of medication and the relatively rare incidence of ARM in the general population. For this reason, I combined our data with those of two similar population-based cohort studies and evaluated the association between medication use and incident ARM in this pooled dataset (Chapter 3.6). Pooling of data, however, introduced an extra potential for misclassification because of inevitable differences between studies in the definition of determinants, confounders, and outcome, as well as differences in the indication for medication use.

Incidence calculation

Incidence is the number of new patients within a defined period of time.[160] It can be expressed either as a percentage of a fixed group (closed cohort) within a specified period, which we call cumulative incidence, or as the number of new patients within a pool of 'person-time', which is called incidence rate. Person-time is the follow-up time, e.g. one year, of one participant at risk of the

disease, and can be summed up irrespective of the number of participants. For example, 100 persons followed for 2.5 years is equal to 50 persons followed for five years. This quantity is used when the population is not closed but consists of different persons over time, or when the time of follow-up varies among subjects in a closed cohort. The latter is true for the Rotterdam Study. The time between baseline and follow-up examination was not exactly the same for each participant. Moreover, there were two follow-up examinations after a median period of two and 6½ years, respectively, and not every subject participated in both. To adjust for these differences in follow-up time, I determined for each person the person-time, added these up and calculated incidence rates. To calculate age-specific incidence rates, each person's contribution of follow-up time to the successive age-categories was determined. So, one person could contribute person-time to different age-categories.

The interpretation of incidence rates is difficult and direct clinical application, e.g. to determine the risk of an individual patient, is not possible. Therefore, I derived from the incidence rate the cumulative incidence with the help of the exponential formula.[160] Cumulative incidence is expressed as percentage with a specified time of follow-up, e.g. five years, and can be readily interpreted as a five-year risk, conditional on survival.

With a disease so intimately correlated with age, an overall estimate of ARM incidence is of not much value and an age-specific incidence is more appropriate. The range of age-categories is arbitrary, but categories of five years are custom. However, the procedure to determine the age-specific incidence in a cohort study is not fully established. If the exact time of disease onset is known, there is no doubt in what age-category that person should be classified. With a slowly progressing disease like ARM there is no clear starting point. It is therefore not possible to determine the person's age at the time of incidence. Moreover, when there is no continuous monitoring of the study population, the age of onset has to be determined retrospectively. Obviously, the disease has started after the baseline examination when the clinical disease was not present and before the follow-up examination when the diagnosis was made. A conventional solution to this problem is to set the time of onset halfway between these two examination dates. This is an accurate estimate only if the age-dependent increase in incidence is linear. For most diseases of the elderly an exponential increase has been described. A statistical model should be used to derive the best estimate of the incidence per age-category. Assuming an exponential increase in incidence with age, a simple alternative would be to set the date of onset at ¾ of the follow-up period. Finally, one might assume that the date of disease onset is similar to the date of diagnosis at the follow-up exam. The age-specific incidence curves resulting from these three assumptions are plotted in Figure 5. It shows that the distribution of incidences over the age-categories varies, with the largest shift in the highest age-category. The described problem is not confined to age-specific incidence rate, but is also involved in cumulative incidence. The

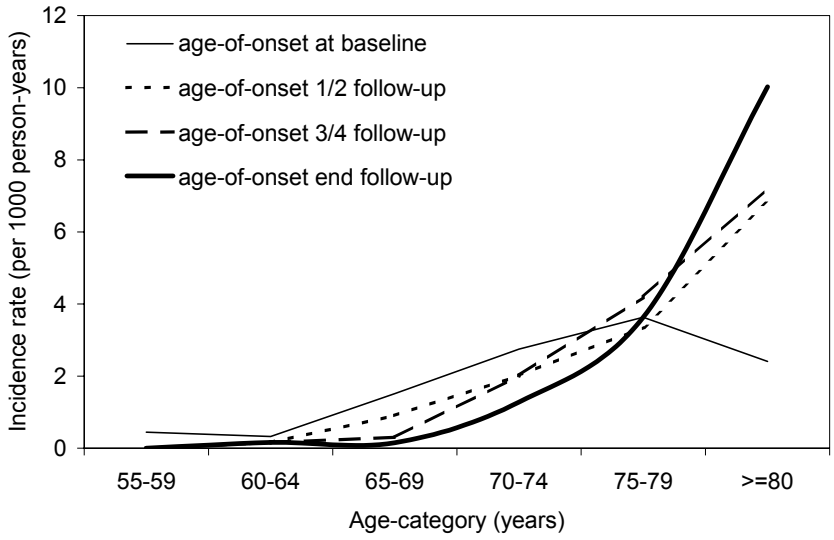


Figure 5 *Age-specific incidence rate of age-related macular degeneration with different assumptions for the age of onset.*

methodology of age-specific incidence assessment in a cohort without continuous monitoring should be further developed and the validity of the assumptions mentioned investigated.

In our incidence study, I adopted the last assumption and took the age of diagnosis as the age of disease onset. The reason was pragmatic, but one should be aware that the true incidence curve is slightly to the left of the one presented and that the incidence estimates at old age are too high.

Definition of ARM

Researchers can take advantage of the fact that the fundus is easily accessible for the quantification of pre-symptomatic stages of. However, it is not self-evident when normality ends and ARM begins. There is broad consensus on the definition of end-stage ARM (AMD). In contrast, the early fundus signs of ARM form a continuum and evolve from scarcely visible small hard drusen to extensive areas of confluent drusen with pigment abnormalities. The prevalence of small hard drusen is very high, up to 40% in persons over 40 years of age.[209] For this reason did the International Epidemiological Study Group not consider them as part of ARM.[24] In their paper from 1995, this group did not come to a clear definition of early ARM. One reason was the lack of incidence data to determine the prognostic value of the separate ARM signs for the development of AMD.

In 2000, Klaver et al. proposed a system of five mutually exclusive stages to quantify the progression of ARM.[98] Definitions and photographic examples of these stages are presented in Chapter 2.1. The next step was to

define a cut-off point that would mark the incidence of early ARM. Based on the results of our study on the risk and natural course of ARM in which we assessed the risk of AMD for all signs of early ARM separately as well as for the stages of ARM, we decided to draw the line of early ARM between stage 1 and 2.[200] The 5-year risk of AMD shifted from 0.9% for stage 1 to 7.8% for stage 2. Moreover, the overall prevalence of stages 0 and 1 combined ranged from 97% for persons aged 55-64 years to 78% for those aged over 85 years. We defined early ARM as the presence of at least soft distinct drusen with pigment changes. Although there is no international consensus at this moment on the definition of early ARM, the definition we used is rather similar to that of two other large cohort studies, the Beaver Dam Eye Study and the Blue Mountains Eye Study.[116, 181]

In most studies, I included early ARM in the incident outcome measure. The assumption was that determinants of incident early ARM are also related to the risk of AMD. One argument for this assumption is the fact that all patients with AMD have early ARM before the onset of AMD, as we showed in Chapter 2.1. The other way around, many persons with early ARM develop AMD. An interesting, though unanswered question is how large this proportion would grow if the time of follow-up were not limited by mortality.

Selection bias

An important threat to the validity of our results comes from selection bias. Its main characteristic is that the relation between determinant and disease is different for those who participate and those who should be theoretically eligible for the study, including those who do not participate.[160] As in all prospective cohort studies, participation to follow-up examinations of the Rotterdam Study decreased over time because of refusal, or loss to follow-up. If this non-participation is correlated with the disease as well as with the determinant, selection bias is introduced. In our study, selective non-response is extra urgent because we did not have continuous monitoring of the cohort and we therefore needed a subject's participation in the examination. Medical files of general practitioners or ophthalmologists are generally not detailed enough to make a reliable diagnosis of ARM, in particular of early ARM. Analysis of baseline characteristics (Chapter 2.1) revealed that persons who refused to participate or were lost to follow-up, were older and less healthy, as indicated by a higher blood pressure, more atherosclerosis, and more often smoking. This indicates that we studied the healthier part of the cohort. Participants were at a lower risk of ARM compared with the total eligible cohort and we therefore underestimated the incidence of ARM in the general population. Also, the healthy cohort will have made it more difficult to demonstrate risk associations, because the distribution of determinants will be different in the study sample as compared with the general population. Selection bias has affected the direction of the associations only if non-participants more often had ARM in combination with the studied determinant. For example, persons with atherosclerosis and ARM would be less likely to

participate in comparison with persons with atherosclerosis and no ARM. It is unlikely that this was the case, especially because early ARM is not symptomatic.

Information bias

Another threat to validity is information bias, which results from misclassification of the outcome or the determinant. Non-differential misclassification, which is random and independent of the factor under investigation, leads to a dilution of the association, making it more difficult to find it. Misclassification of the outcome that is related to the exposure status, or vice versa, is called differential misclassification, and results in a distortion of the findings. This distortion cannot be adjusted for in the analysis. The prospective design of our studies, with the measurement of exposure before the onset of ARM, excludes the possibility of differential misclassification of the determinant. The same holds for missing data caused by failure of equipment, absence of the research assistant, or refusal of the subject to participate in that specific examination. Because these data were collected at baseline, their absence cannot be associated with the incidence of ARM. Differential misclassification of ARM is also very unlikely, because persons who performed the grading of ARM were blinded for exposure status of the cases. Grading of fundus transparencies in a standard, well-established procedure by the same well-trained graders, reduced the potential of non-differential misclassification of ARM. In addition, the graders assessed the incidence of ARM by comparing follow-up photographs with those of baseline, and the principal investigators of two other cohort studies that use the same grading methods, confirmed the diagnosis of incident AMD.

Confounding

Confounding may be considered a confusion of effects.[160] A confounder must be a risk indicator of the disease independent of the determinant under study and must be associated with the determinant. In addition, a confounder should not be an intermediate in the causal pathway between determinant and disease. On can neutralize the effect of a confounder by measuring it carefully and adjusting for it in the statistical analyses. Age is probably the most important confounder in associations with ARM. It is a strong risk indicator of ARM and is associated with all determinants under study. Therefore, age was included in all analyses. Even though gender did not appear to be associated with ARM (Chapter 2.1), it was regarded as a potential confounder by convention. Since refractive error is not related to other ARM determinants such as smoking, blood pressure, and atherosclerosis, we did not consider refraction a confounder in relation to ARM.

Confounding-by-indication is a particular form of confounding which is relevant to the analysis of medication use. It refers to the phenomenon that the relation found between medication use and a disease is representative of an association between a risk indicator for which the medication was prescribed,

and the disease. Confounding-by-indication may be the explanation for the observed association between the use of antihypertensive medication and ARM, because hypertension is a risk factor of ARM (Chapter 3.2). Confounding was also responsible for the observed association between dietary intake of n-6 polyunsaturated fatty acids and ARM (Chapter 3.5). The association disappeared when we adjusted for vitamin E intake, showing that polyunsaturated fatty acids are only linked to ARM because of their mutual relation with vitamin E.

Even though I adjusted for many potential confounders, the possibility of insufficient measurement of confounders, or the presence of unknown confounders should always be considered in observational studies. Residual confounding may for example interfere with the association between intake of antioxidants and ARM, because antioxidant intake is part of a healthy diet and a healthy diet is correlated with many other lifestyle factors. Only randomised trials can minimize the problem of residual confounding.

MAIN FINDINGS

Frequency

The incidence of ARM in the general population was estimated based on 47 persons who developed AMD and 417 who developed early ARM (Chapter 2.1). The incidence of AMD, expressed as five-year risk, increased exponentially with age and ranged from 0.1% for persons aged 60-64 years to 3.4% for those aged 80 years and over. AMD incidence was not only determined by age, but also by the presence of early fundus signs of ARM. The presence of 10 or more hard drusen was associated with a five-year risk of AMD of 3%, while 10 or more large soft drusen carried a risk of 17%. It should be noted that I did not take the competing risk of death into account. Therefore, the presented risk estimates are conditional on survival and will be underestimations of the true incidence. No risk difference was observed between men and women. Also, the incidence and natural course of atrophic and neovascular AMD were remarkable similar.

I conclude that ARM is a slowly progressive disease in which a pattern of changing fundus signs can be detected. Small drusen become larger, more indistinct, more confluent, and they cover a larger area of the macula. The presence of pigment changes is an additional step in this progression. I could not demonstrate a statistically significant difference in the natural course of atrophic and neovascular AMD. However, the data suggest that a neovascular haemorrhage may occur in the presence of relatively few drusen, while atrophic AMD may be the final stage in a retina with more extensive drusen formation. Perhaps in the latter situation the trigger for neovascularisation is not present.

Furthermore, I propose that ARM should be regarded as an acceleration of a normal ageing process in the retina. The age at which drusen form and the extent to which these drusen progress is different between subjects. One might argue that all humans will develop drusen if their life span is infinite.

Determinants

I will discuss the main findings concerning the determinants of ARM in light of the different aetiological pathways that have been proposed.[15] There is evidence that choroidal blood flow is impaired in patients with ARM, suggesting that hemodynamic changes play a role. Several studies in this thesis support a contribution of cardiovascular pathology to the development of ARM. The observed association between elevated blood pressure and atherosclerosis, and increased risk of incident ARM is the most direct support for the cardiovascular hypothesis (Chapter 3.2). I found that per 10 mm Hg increase in systolic blood pressure the risk of ARM increased by 8%. The presence of sub-clinical atherosclerosis, especially in the carotid artery, increased the risk of ARM in a dose-dependent manner. Other indicators of atherosclerosis, including pulse pressure, ankle-arm index, and aorta calcifications, were also associated with an increased risk of ARM. Another finding in support of a cardiovascular role in ARM aetiology was the increased mortality among people with ARM, including the early stages (Chapter 4). Our study showed that cardiovascular risk factors, including hypertension, atherosclerosis, and smoking, were responsible for the relationship between ARM and mortality, implicating that cardiovascular factors are positively associated with ARM. Finally, I found that persons using blood pressure lowering medication were at an increased risk of developing early ARM (Chapter 3.6). This finding also provides indirect evidence for an association between hypertension and ARM. I could not demonstrate an association between ARM and serum total cholesterol, which is an established risk factor for coronary heart disease (Chapter 3.3). Also, dietary intake of fatty acids that are known to promote atherosclerosis was not associated with incident ARM in our cohort. In summary, our data provide evidence for an association between vascular pathology and ARM. Moreover, this association is most likely a causal one, considering the temporal relationship between the presence of hypertension or atherosclerosis and ARM development.

Another theory of ARM aetiology implicates oxidative stress at the level of the retinal pigment epithelium.[19] Oxidative stress refers to cellular damage due to reactive oxygen intermediates. The role of oxidative stress in the pathogenesis of ARM is biologically plausible, but clinical and epidemiological proof is still lacking. To further test this theory, I studied dietary intake of antioxidant micronutrients, including carotenoids, vitamins, and trace elements, in relation with the risk of ARM. A strong protective effect was observed of a high intake of vitamin E and zinc, two antioxidants that are present in the human retina in large quantities. An inverse association between intake of α- and β-carotene and lutein/zeaxanthin did not reach statistical significance. These findings strongly support a role of oxidative stress in the aetiology of ARM.

It is difficult to put the association between refractive error and ARM into an aetiological perspective. In our cohort, hyperopia increased, whereas

myopia decreased the risk of ARM. This association was found for both early and late ARM. Before interpreting this finding, a few pitfalls should be considered. Misclassification of drusen in a light myopic fundus may explain the association with early ARM, while a wrongful diagnosis of myopic macular degeneration instead of neovascular ARM may explain the association with late ARM. If we suppose that the relationship is true, there are several explanations possible. The greater thickness and rigidity of the sclera of hyperopic, compared with myopic eyes, may result in hemodynamic changes. The greater retinal area of elongated myopic eyes may result in a lower burden of ultraviolet or visible light per square micrometer. However, given the speculative nature of these explanations, I cannot link the observed association, if true, with an overall hypothesis on the aetiology of ARM.

CLINICAL RELEVANCE

Are the studies described in this thesis relevant to clinical practice? I think so. The assessment of an individual's risk of end-stage ARM can directly be applied to clinical practice. For this purpose, I provided an insert with photographic examples of the ARM stages in a convenient format. In combination with Table 2.1.5, one can estimate the five-year risk of end-stage ARM of a patient based on age and fundus characteristics. Patients at high risk may be advised to quit smoking. Cigarette smoking is the strongest modifiable risk factor of ARM at this moment.[38] In addition, based on Chapter 3.4, it may be justifiable to give a dietary advice. Our results suggest that an increase in the consumption of food products rich in vitamin E and zinc may protect against ARM. The investigators of the Age-Related Eye Disease Study proposed a similar policy based on a trial with high-dose antioxidant supplementation. They identified a subgroup of patients with early ARM who may be eligible for these supplements. Considering the potential for adverse effects of the high doses of micronutrients, especially zinc, a modification of dietary intake would be preferable.

The association between blood pressure and ARM is also of clinical relevance. The increased risk of ARM associated with a systolic blood pressure above 160 mm Hg suggests that blood pressure lowering may help in the prevention of ARM. The clinical consequence would be that patients with early ARM should have their blood pressure measured and be treated accordingly. However, timing and target of blood pressure lowering for the purpose of ARM prevention are unknown. As long as experimental data are not available, recommendations on blood pressure lowering in patients with ARM are speculative.

SUGGESTIONS FOR FUTURE RESEARCH

As just mentioned, the potential effect of blood pressure lowering on the risk of ARM may be the subject of a clinical trial. A well-defined group of people with early ARM may be randomised to standard ophthalmologic care or

standard care with treatment of elevated blood pressure according to established protocols. Endpoint of such a trial would be progression of early ARM severity or progression to end-stage ARM.

To further investigate the exact role of cardiovascular disease in the pathophysiology of ARM, one has to measure the local vascular changes in the eye. Epidemiological studies have focused on either cardiovascular risk factors or atherosclerosis of the large systemic blood vessels. Only very few studies have measured ocular blood flow in relation to ARM. Possible techniques to assess ocular blood flow include scanning laser ophthalmoscopic angiography, laser Doppler flowmetry, and traditional fluorescein or indocyanine angiography.[69, 154] Flow in the ophthalmic artery can be determined by colour Doppler ultrasound imaging. For larger, and preferentially population-based studies, these techniques are difficult to implement. Case-control studies cannot determine a temporal relation between blood flow and ARM. My suggestion would be to use the scanning laser ophthalmoscope in an ongoing cohort study and to study the incidence of early ARM. This allows for causal. Another promising technique is retinal vessel measurements on pre-existing fundus transparencies. Diameters of retinal arterioles and venules have been shown to reflect long-lasting hypertension and to predict cardiovascular events.[81, 229] Although changes in retinal vessel diameters may represent micro vascular damage, a relation with the condition of the choriocapillaris has still to be established.

Testing of the oxidative stress hypothesis in relation to ARM is a major challenge for epidemiological studies. Although dietary intake will reflect the long-term exposure to specific nutrients, provided that diet does not change much over a life time, serum levels of nutrients are objective and will show less measurement error.[224] Therefore, large prospective population-based studies on the association between serum levels of antioxidants, such as vitamins C and E, carotenoids, and trace elements, are awaited. The Pathologies Oculaires Liees a l'Age study tested levels of antioxidant enzymes, such as plasma glutathione peroxidase and erythrocyte superoxide dismutase.[48] They reported a nine-fold increase in late ARM prevalence. Confirmation of this association has not been reported so far, but the association certainly deserves further study. A potential determinant of ARM that is related to oxidative stress is macular pigment density. Macular pigment is thought to protect against local oxidative stress, and a few small studies have suggested that its concentration is associated with the risk of ARM.[20] However, no association was found in a cross-sectional analysis of the Rotterdam Study.[22] Prospective studies are needed to establish the relationship between macular pigment and risk of ARM.

The recent interest in the role of immune processes in the pathology of ARM should stimulate epidemiologists to study this relationship in the general population.[66] Markers of immune activation that can be measured in a large group of people include acute phase proteins such as C-reactive protein, fibrinogen, ceruloplasmin, and α1-antichymotrypsin. Another candidate

marker of inflammation is erythrocyte sedimentation rate. However, these markers are not disease specific and may reflect many processes such as smoking, overweight, and co-morbidity, making the interpretation of a possible association difficult. More selective markers of immune status include cytokines, such as interleukin-1 and interleukin-6. Another way of evaluating the association between inflammation and ARM is to analyse the impact of anti-inflammatory medication, in particular the non-steroidal anti-inflammatory drugs. If an activated immune system promotes the development of ARM, a protective effect of non-steroidal anti-inflammatory drugs is to be expected.

CONCLUSIONS

Considering the results of all studies in this thesis, I postulate that the extent of oxidative stress at the level of the retinal pigment epithelium is central in the aetiology of ARM, while many endogenous characteristics, including genetic predisposition, as well as exogenous provocations contribute to a person's susceptibility to oxidative stress. The level of oxidative stress is a balance of burden and defence. The physiological burden of oxidative stress in the retina is exceptionally high due to its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, its intense exposure to visible light, and its concentration of many photosensitisers, including lipofuscin.[19] The defence of the retina against oxidative damage consist of antioxidants, such as glutathione, vitamins C and E, and carotenoids, as well as repair mechanisms of oxidized proteins. Both sides of the balance will be influenced by many factors, both genetic and environmental. An example of genetic predisposition includes genetic polymorphisms that determine the concentration and functionality of antioxidant enzymes. In addition to genes, ocular factors such as refractive error, condition of the retinal blood vessels, and cataract extraction, contribute to the risk of ARM. Finally, behaviour of the individual determines the intensity of light exposure to the eyes, intake of antioxidant nutrients, and the extra burden of oxidative stress by smoking. The combination of all these determinants decides the age of onset, progression rate, and final stage of ARM.

Epilogue

Who will benefit from this thesis? For present patients with bilateral end-stage AMD, I think that there is no direct benefit, because this thesis did not study therapeutic interventions. Indirectly, however, the thesis contributes to the elucidation of the aetiology of AMD, and this knowledge will help in the development of new therapies in the future. For patients with AMD in one eye, or with the early stages of AMD, the results of the described studies offer the potential of preventing the blinding end-stage of AMD. In particular, our results suggest that lowering of high systolic blood pressure and increase in dietary intake of vitamin E, zinc, and possibly β-carotene, may decrease the risk of AMD. These measures can directly be applied in practice. Ideally, a clinical trial should be performed to confirm our observational findings. Before these complex and expensive trials will be realized, persons with early stages of AMD may want to increase their intake of the above-mentioned antioxidants. For them, I have included a recipe at the end of this thesis.

For the scientific community, I think that this thesis provides a new stepping-stone to the unravelling of the aetiology of AMD. Inherent to the observational nature of the studies, no definite evidence can be presented. Still, the prospective design allows for causal inference. The contribution of a large population-based study such as the Rotterdam Study may be to confirm or rebut the results of smaller, clinic-based studies. Our studies on atherosclerosis, dietary antioxidants, and cholesterol-lowering medication use are examples of this. Discovering new risk factors is another opportunity for population-based studies, as exemplified by our studies on medication use and APOE. Finally, studies on the incidence and natural course of AMD are only possible within a large sample of the general population.

For myself, this doctoral thesis was a valuable scientific lesson and a very pleasant adventure.

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Summary

Age-related maculopathy (ARM) is a degenerative disease of the retina and the leading cause of incurable blindness and visual impairment in industrialised countries. The cause of the disease is largely unknown and options for therapy and prevention of ARM are limited. The aim of the studies described in this thesis was to determine the risk of ARM in the general population, and to analyse the association of endogenous and exogenous factors with ARM development. The results of these studies will hopefully contribute to the understanding of the aetiology of ARM and provide clues for its prevention. Most studies were performed within the Rotterdam Study, a population-based cohort study among 7983 persons of 55 years and older.

Chapter 1.1 is a short introduction to the thesis, followed by a review of previous research on the epidemiology of ARM in **Chapter 1.2**. Subsequently, the research objectives are defined in **Chapter 1.3**.

In Chapter 2.1, we assessed the frequency of new cases (incidence) of ARM and described the natural course of the early stages. The incidence of ARM was strongly determined by age and the presence of early retinal abnormalities (drusen), but not by gender. The 5-year risk of late ARM ranged from 0% for persons with no drusen irrespective of age to 42% for persons 80 years or older with indistinct drusen and pigment changes. We observed that the progression of early ARM followed a distinct course and accelerated with increasing age.

Chapter 2.2 describes a study in which we compared the reliability of digital retinal photography with that of traditional 35-mm film for the grading of ARM in an epidemiological setting. The agreement between both imaging techniques was good to very good. We concluded that, considering the practical advantages and low cost per image, digital photography might serve well in epidemiological studies.

In **Chapter 3.1**, we studied refractive error of the eye in relation to the risk of ARM. We found that hyperopia (long-sightedness) increased, whereas myopia (short-sightedness) decreased the risk of both early and late ARM. The

cause of this association is still unknown, but we provide some possible explanations.

The much-debated relationship between atherosclerosis and ARM is studied in **Chapter 3.2**. In the baseline phase of the Rotterdam Study, measures of sub-clinical atherosclerosis, such as carotid wall thickness and plaques in the carotid artery were collected. We found that these direct measures, as well as indirect indicators of atherosclerosis, were in a dose-dependent way associated with an increased incidence of ARM. In addition, elevated systolic blood pressure was positively associated with incident ARM. These data provide the strongest evidence so far that atherosclerosis is involved in the development of ARM.

In **Chapter 3.3**, a study on cholesterol and ARM is described. We confirmed previous suggestions that high-density lipoprotein cholesterol is associated with an increased risk of ARM. In addition, we showed that the strength of this association is altered by variations in the apolipoprotein E genotype. An explanation for this relation is still unknown.

The effect of diet on the risk of ARM is investigated in **Chapters 3.4** and **3.5**. At the start of the Rotterdam Study, participants were asked about their dietary habits and subsequently the average intake of specific nutrients was calculated for each participant. We found that a high dietary intake of vitamin E and zinc, which are important antioxidants in the eye, was associated with a lower risk of ARM. Furthermore, we observed an inverse association between intake of polyunsaturated fatty acids and ARM. However, this association disappeared after correcting for vitamin E intake. Because intake of the fat-soluble vitamin E is correlated with consumption of unsaturated fat, and vitamin E lowers the risk of ARM, the observed association could be explained (is confounded) by vitamin E intake.

In **Chapter 3.6**, we analysed the use of many different types of medication and ARM. We combined three similar cohort studies into one dataset. Despite the large statistical power of this analysis, only a few weak associations were found. The increased risk of ARM among users of antihypertensive medication, in particular beta-blockers, was probably the result of the relationship between blood pressure and ARM.

Chapter 3.7 describes a study on the effect of cholesterol-lowering medication on ARM. Previous studies have suggested that these drugs protect against ARM. We measured the cumulative exposure to cholesterol-lowering drugs, including statins, and did not find any association with the incidence of ARM.

In **Chapter 4**, we asked ourselves whether ARM and other age-related eye disorders are related to mortality. This question is interesting because it may give us insight into risk factors of ARM that are also related to mortality. We indeed found that ARM was associated with an increased mortality rate, and that this relationship disappeared after correction for blood pressure and indicators of atherosclerosis. This analysis provides further evidence for a role of cardiovascular factors in the pathogenesis of ARM.

Finally, in **Chapter 5**, the methodology of the studies, the main findings and their clinical relevance, suggestions for further epidemiological research, and final conclusions are discussed.

Samenvatting

Ouderdoms maculadegeneratie (OMD) is een degeneratieve aandoening van het netvlies. Zij is de belangrijkste oorzaak van ongeneesbare blindheid en slechtziendheid in geïndustrialiseerde landen. De oorzaak van de ziekte is grotendeels onbekend en de mogelijkheden voor behandeling en preventie zijn zeer beperkt. Het doel van het in dit proefschrift beschreven onderzoek was om het risico op OMD vast te stellen en de invloed van endogene en exogene factoren hierop te bestuderen. Het onderzoek zal hopelijk bijdragen aan een beter begrip van de oorzaak van OMD en aanwijzingen geven voor de behandeling en preventie. De meeste studies in het onderzoek zijn uitgevoerd binnen het Erasmus Rotterdam Gezondheid en Ouderen onderzoek, een langlopend bevolkingsonderzoek onder 7983 personen van 55 jaar en ouder.

Hoofdstuk 1.1 is een korte introductie over OMD. Een overzicht van het bestaande onderzoek naar de epidemiologie van deze aandoening wordt gegeven in **Hoofdstuk 1.2**. Vervolgens worden in **Hoofdstuk 1.3** de doelstellingen van het onderzoek gedefinieerd.

In **Hoofdstuk 2.1** bepalen we de frequentie van nieuwe ziektegevallen (incidentie) en beschrijven we het natuurlijk beloop van de voorstadia van OMD. In dit onderzoek werd de incidentie sterk bepaald door leeftijd en aanwezigheid van vroege netvlies afwijkingen (drusen). Het geslacht bleek niet van invloed. Het vijf-jaars risico op de late vorm van OMD varieerde van 0% voor personen zonder drusen onafhankelijk van leeftijd, tot 42% voor personen van 80 jaar of ouder met indistincte drusen en pigmentveranderingen. We zagen dat de progressie van de voorstadia van OMD een vast patroon vertoonde en versnelde met het toenemen van de leeftijd.

Hoofdstuk 2.2 beschrijft een studie waarin we de betrouwbaarheid van digitale fotografie vergelijken met die van de traditionele 35-mm fotografie voor het graderen van OMD in epidemiologische studies. De overeenkomst tussen beide beeldvormende technieken was goed tot zeer goed. Gezien de praktische voordelen en lage kosten per foto lijkt digitale fotografie een betrouwbaar alternatief in epidemiologische studies naar OMD.

In **Hoofdstuk 3.1** bestuderen we de refractie van het oog in relatie tot OMD. We vonden dat het risico op zowel de vroege als late vormen van LMD was verhoogd bij hypermetropie (verziendheid), terwijl dit risico was verlaagd bij myopie (bijziendheid). De oorzaak van dit verband is helaas nog onbekend, maar we geven een aantal mogelijke verklaringen.

Het omstreden verband tussen atherosclerose en OMD wordt bestudeerd in **Hoofdstuk 3.2**. In de beginfase van de Rotterdam Studie zijn verschillende maten van atherosclerose verzameld, zoals wanddikte van de halsslagader en de aanwezigheid van atherosclerotische plaques. Wij vonden nu dat deze maten, evenals indirecte indicatoren voor atherosclerose, geassocieerd waren met een hoger risico op OMD. Tevens zagen we dat een hoge systolische bloeddruk gepaard ging met een hoger risico op OMD. Deze resultaten leveren het beste bewijs tot nu toe dat atherosclerose betrokken is bij het ontstaan van OMD.

In **Hoofdstuk 3.3** wordt een studie beschreven naar cholesterol en OMD. We konden eerdere suggesties bevestigen dat een subtype (HDL) cholesterol geassocieerd is met een hoger risico op OMD. Daarnaast hebben we aangetoond dat de associatie beïnvloed wordt door variaties in een gen dat betrokken is bij het transport van cholesterol.

De invloed van voeding op het risico op OMD is onderzocht in de **Hoofdstukken 3.4** en **3.5**. Deelnemers aan de Rotterdam Studie is gevraagd naar hun voedingsgewoonten waarna hun gemiddelde inname van specifieke bestanddelen is berekend. Wij vonden dat hoe groter de consumptie van vitamine E en zink, belangrijke antioxidanten in het oog, hoe lager het risico op OMD was. Verder zagen we een omgekeerd evenredig verband tussen inname van meervoudig onverzadigde vetzuren en het ontstaan van OMD. Dit verband verdween echter na correctie voor vitamine E inname. Omdat consumptie van het vetoplosbare vitamine E gecorreleerd is met de inname van onverzadigde vetzuren, en vitamine E het risico op OMD verlaagt, kon het eerder genoemde verband verklaard worden door vitamine E.

In **Hoofdstuk 3.6** hebben we gekeken of het gebruik van verschillende soorten geneesmiddelen invloed heeft op het ontstaan van de voorstadia van OMD. Hiertoe hebben we de gegevens van drie vergelijkbare bevolkingsonderzoeken gecombineerd om de statistische mogelijkheden te vergroten. Desondanks vonden we slechts enkele zwakke associaties. Het verhoogde risico op OMD onder gebruikers van bloeddrukverlagende middelen is waarschijnlijk een aanwijzing voor een verband tussen hoge bloeddruk en OMD.

Hoofdstuk 3.7 beschrijft een studie naar het effect van cholesterol verlagende geneesmiddelen op OMD. Eerdere studies suggereerden dat deze medicijnen mogelijk beschermen tegen OMD. Wij hebben het cumulatief gebruik van cholesterol verlagers gemeten over een lange periode en vonden geen enkel verband met het risico op OMD.

In **Hoofdstuk 4** vroegen we ons af of OMD en andere leeftijdsgebonden oogaandoeningen gepaard gaan met een verhoogd risico op

sterfte. Deze relatie is interessant omdat het ons aanwijzingen kan geven over factoren die zowel met OMD als met sterfte samenhangen. We vonden inderdaad dat personen met OMD een verkorte levensduur hadden, maar dat deze relatie verdween na correctie voor bloeddruk en atherosclerose. Deze studie levert verder bewijs dat cardiovasculaire factoren een rol spelen bij het ontstaan van OMD.

Tot slot bespreek ik in **Hoofdstuk 5** de methodologie van de studies, de belangrijkste bevindingen en hun klinische relevantie. Verder geeft ik suggesties voor verder epidemiologisch onderzoek en kom ik tot een conclusie.

Dankwoord

Heel graag wil ik iedereen die mij direct of indirect heeft geholpen bij het schrijven van dit proefschrift hiervoor bedanken. In het bijzonder:

Professor De Jong en professor Hofman, mijn promotoren. Paulus, je markante persoonlijkheid en je originele manier van begeleiden hebben bijgedragen aan de kwaliteit van het onderzoek, maar evenzeer aan het plezier waarmee ik daar aan heb gewerkt. Ik heb veel van je geleerd en je niet aflatende aandacht voor detail en werklust hebben mij altijd alert gehouden, zelfs op het strand van Fort Lauderdale. Bert, je uitgesproken bevlogenheid in alles wat je doet en het elan dat je aan de afdeling geeft maakten mijn promotie tot een inspirerende tijd. Bedankt voor de levendige discussies en je vele aanmoedigingen.

Professor Moore, professor Van Rij and professor De Zeeuw, members of the 'kleine commissie'. I am grateful for your review and approval of the manuscript. Professor Van Rij wil ik daarnaast hartelijk danken voor het in mij gestelde vertrouwen door mij toe te laten tot de opleiding. Ik verheug me zeer op de komende jaren onder uw leiding. Professor Stricker en dr. Schlingemann dank ik voor het zitting nemen in de 'grote commissie'. Bruno, jou wil ik natuurlijk ook bedanken voor je grote betrokkenheid bij het HDL vraagstuk. Je kritische, maar altijd optimistische commentaar en de discussies vrijdagavond als iedereen de afdeling al had verlaten heb ik erg gewaardeerd.

Hans Vingerling en Caroline Klaver, mijn illustere voorgangers in het ERGO-AMD onderzoek. Ik wil jullie bedanken voor jullie hartelijkheid en interesse, in en buiten het onderzoek. Jullie zijn fantastische begeleiders en ik ben blij dat we ook de komende jaren zullen samenwerken. Hans, je humor en de wijze waarop je, vaak na een paar weken, met nieuwe en originele oplossingen kwam waren erg stimulerend. Caroline, ik heb heel veel gehad aan je inzicht, enthousiasme en perfectionisme. Het is een eer dat jij mijn paranimf wilt zijn en ik hoop dat we nog vaak langs de Rotte of in Central Park zullen skaten.

Co-authors I have not mentioned yet, professors Klein, Sandy Tomany, professor Mitchell, Jie Jin Wang, professor Cumming, professor Van Duijn, dr. Mulder, and dr. Witteman, thank you for your valuable comments.

Corina Brussee, Ada Hooghart, Kamran Ikram, Petra Poorts-Borger, Simone de Voogd, en, sinds een paar weken Raph de Haas, Siamand Jaf en Dolinda Pottuit, mijn kamergenoten gedurende langere of kortere tijd. Dank jullie wel voor de goede sfeer op de kamer en de positieve reaktie op mijn muziekkeuze. Ada en Corina, jullie zijn de betrouwbare steun van onze groep. Jullie gezelligheid en heldere commentaar op van alles en nog wat zal ik missen. Petra, bedankt voor de leuke tijd dat wij met z'n tweeën de ooggroep vormden. Kamran, Simone en, op de valreep, Shamila, succes met de resterende promotietijd. Ik kijk uit naar jullie boekjes. Dolinda, bedankt voor de altijd verse koffie. Raph, mooie CD van Schnittke! Siamand, zullen we een keer zeilen met je nieuwe boot? Jacqueline Willemse, Caroline Hulsman, Roger Wolfs, Raan Ramrattan, en Gerard de Bruyne wil ik bedanken voor de goede samenwerking en de vrolijke bezoeken aan de oogkamer.

Henning Tiemeier, ik vind het geweldig dat we na een bijna gelijktijdige start op de afdeling elkaar nu (hebben) kunnen bijstaan in het afronden van onze promoties. Ik hoop dat we ook in de toekomst nog vaak discussie zullen voeren en samen het Holland Festival bezoeken. Bedankt ook voor je gründliche en opbouwende kritiek op mijn teksten. Je promovendi zullen een pittige begeleider krijgen. Marianne Engelhart en Irene van der Meer, bedankt dat ik gebruik heb mogen maken van de door jullie uitgedachte methode voor fat intake analyse en atherosclerosis composite score. Irene, het was leuk om de laatste promotie perikelen met je te kunnen delen.

Alle medewerkers op het ERGO-centrum, Lydia Buist, Pauli Eldik-van Helleman, Henriette Ensing, Trudy Groenewegen-Hoogeveen, Inge Haumersen, Saskia Hoornweg, Anneke Korving, Edith Nolet, Anneke Palsma, Toos Stehman, bedankt voor jullie vele en nauwkeurige werk waarop alle studies zijn gebaseerd. En daarnaast voor de gezelligheid tussen de onderzoeken door.

De mensen op de afdeling die ik altijd met een vraag mocht storen en onverstoorbaar antwoord gaven: Marga van den Bergh, Petra van Rikxoort, Marjolein Kasi, Marti von Stein, Yolanda Bekker, Nano Suwarno, Frank van Rooij, Erik Neeleman, René Vermeeren, Bettina Hansen, Maria de Ridder, Rachid el Galta en Lidia Arends.

Alle deelnemers aan ERGO, dankzij uw medewerking is ons onderzoek mogelijk. Met name dank voor uw bereidheid die prikkende oogdruppels te ondergaan.

Familie en vrienden, op de achtergrond maar essentieel; mijn fantastische ouders, Max en Eef van Leeuwen-Sauer, bedankt voor jullie onvoorwaardelijke steun en het meedenken op alle vlakken; Matthijs, broertje, bedankt voor het delen van je grote ervaring in het 'opmaken' van een proefschrift; tante Janna, U als MD-patiënt gaf mij een zeer persoonlijke motivatie voor dit onderzoek. Met

name dank voor uw nuchtere vragen en opmerkingen (“Sinds ik die medicijnen slik zijn mijn ogen achteruit gegaan”); mijn schoonouders en Monika en Just, die alle begrip hebben voor promotie drukte en bij wie de deur altijd open stond om na een lange dag te ontspannen met een bordje sajoer of groene currie. Van mijn vrienden wil ik hier de leden van mijn strijkkwartet noemen, Daphne Gerrits, Lucie van Rooijen en Harmen Jan Holman, voor de afleiding in het weekend met muziek en soep. Het gaf even verwarring bij collega’s toen ik vertelde dat ik zondag ging kwartetten, maar al snel werd het “Heb je weer gekwartet?”

Tijdens het werk luisterde ik vaak naar muziek, via de radio of de CD speler in mijn computer. Maar het beste kon ik werken als ik op de achtergrond Ilona hoorde studeren, op de Ciaccona van Bach of het vioolconcert van Beethoven. Haar passie en perfectionisme in de muziek hebben mij al 15 jaar geïnspireerd. Wat Ilona voor mij betekent kan ik niet in woorden vatten. Binnenkort zullen we geen duo maar een trio vormen en hoor ik hele nieuwe klanken op de achtergrond. Jouw ongeduldige getrappel was de beste prikkel om mijn proefschrift nu af te ronden.

Biography

Redmer van Leeuwen was born in Groningen, the Netherlands, on 29 December 1970. He attended the Christelijke Scholengemeenschap (now called Hondsrug College) in Emmen before moving to Rotterdam in 1989 to study Medicine at Erasmus University Rotterdam. The same year he studied violoncello at the Conservatory of Utrecht. During his studies he was an active member of several student organisations, participated in different research projects, and was student assistant at the Departments of Anatomy and Pharmacology. He passed the United States Medical Licensing Examination in 1996, and in 1997 obtained his medical degree. He spent one year in the United States, working at the Geisinger Hospital, Danville Pennsylvania, and at the Laboratory of Neuroimmunology of Columbia University, New York, headed by Prof. N. Latov. After his return to the Netherlands he worked at the Department of neurosurgery of the Free University Medical Centre in Amsterdam, headed by Prof.dr. W.P. Vandertop. In 1999 he returned to his Alma Mater to start the work described in this doctoral thesis under supervision of Prof. dr. P.T.V.M. de Jong and Prof.dr. A. Hofman at the Department of Epidemiology and Biostatistics. He obtained a Master of Science degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences in 2002. August 2003 he started a residency in ophthalmology at the Department of Ophthalmology of Erasmus MC, headed by Prof. dr. G. van Rij.

Redmer is married to Ilona Sie Dhian Ho, violinist, and in September they expect their first child.

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Recept met AMD-beschermende ingrediënten

Spinazie taart

Ingrediënten:

8 plakjes bladerdeeg	(zink, vitamine E)
600 g spinazie	(luteine, β-caroteen)
250 g champignons	
1 rode paprika	(β -caroteen, vitamine E)
200 g maiskorrels	(luteine/zeaxanthine)
250 g (oude) kaas	(zink)
3 eieren	(luteine/zeaxanthine, vitamine E)
40 g bloem	(zink)
olijfolie	(vitamine E)

zout, peper, basilicum, oregano, paneermeel

Bakblik (Ø 23 cm) invetten, bekleden met plakjes bladerdeeg en bodem bestrooien met paneermeel. Oven voorverwarmen op 200º. Spinazie koken met zout en daarna vocht zoveel mogelijk afgieten. In olijfolie stukjes champignons, paprika, en mais bakken. Ruime hoeveelheid zout (mits er geen hoge bloeddruk bestaat), peper, basilicum en oregano toevoegen. Mengsel bij de spinazie voegen en opnieuw vocht afgieten. Eieren, blokjes kaas en zelfrijzend bakmeel door mengsel roeren. Geheel in bakvorm gieten, bedekken met plakjes kaas, laatste plakje bladerdeeg erop leggen en de taart dichtvouwen. Bakken gedurende 75 minuten bij 200º. Oven/magnetron combinatie is ook geschikt.