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Nutrition and Ocular Disease in an Older Australian Cohort

The Blue Mountains Eye Study

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Preface

This thesis describes the relationship between newer dietary factors and ocular disease in an older Australian cohort. The thesis is in 7 parts and 13 chapters. Part 1 provides an overview of the current literature relating different aspects of diet to the ocular diseases most commonly responsible for blindness, cataract, age-related macular degeneration and retinal microvascular disease. Part 2 describes the population studied in this thesis and methods used to define cases with ocular disease and analyse the nutritional status of the population. Parts 3 to 5 describe the findings from this thesis in relation to retinal microvascular disease, age-related macular degeneration and cataract. Part 6 discusses the impact of diet on overall visual impairment. Part 7 concludes the thesis and discusses the implications and future directions arising from this thesis.

The thesis is constructed as a series of publications and manuscripts, thus there is repetition of some aspects of the methods and results.

The thesis describes the full-time involvement (2005 to 2007) and part-time involvement (2008 to 2009) of the candidate in the Blue Mountains Eye Study. The Blue Mountains Eye Study (BMES) is a longitudinal population-based cohort of visual impairment and common eye diseases in an older Australian population.

Abstract

Objectives: To assess the relationship between a variety of dietary factors and retinal microvascular signs, age-related macular degeneration and cataract in an older Australian cohort.

Methods: An urban, predominantly Caucasian population-based sample aged 49 years or older in Blue Mountains region, west of Sydney, Australia was examined over a 10-year period. At baseline in 1992-4, 3654 participants (82.4% response) were examined. Participants were then examined at 5-year intervals; 2335 (75.1% of survivors) at the second examination in 1997-9, and 1952 (76.5% of survivors) at the third in 2002-4.

At each visit, participants underwent a detailed interview which included a 145-item, validated food frequency questionnaire and questions regarding demographic information, medical and social history and visits to eye care services. An examination was conducted to measure vision, height, weight, blood pressure and to gather fasting blood samples. Visual acuity was measured before and after subjective refraction using a LogMAR chart. Visual impairment was defined if best-corrected visual acuity in the better eye was 6/12 or worse.

Slit-lamp and retroillumination lens photographs were taken for cataract grading, and stereoscopic retinal photographs were taken, after pharmacologic pupillary dilatation, for assessment of retinal microvascular signs and age-related macular degeneration (AMD). Grading of retinal signs, AMD and cataract was based on the Wisconsin system. Participants

who died were identified by linkage with the Australian National Death Index. All statistical analyses were conducted using the Statistical Analysis System software (SAS).

Results:

Retinal microvasculature findings

By accounting for the correlation between retinal arteriolar and venular calibre using the residual method, analysis of retinal arteriolar and venular calibre independent of the fellow vessel component was demonstrated. This methodology was subsequently used in the thesis.

A 31.7% prevalence of the retinal arteriolar light reflex was found in the baseline population (with 28.8% graded as mild retinal arteriolar light reflex and 2.9% as marked). Prevalence of mildly enhanced light reflex decreased with increasing age ($p_{(trend)}<0.0001$) and persons with cataract were less likely to have mildly enhanced light reflex (odds ratio, OR, 0.74, 95% confidence interval, CI, 0.64-0.87). Markedly enhanced light reflex was significantly associated with mean arterial blood pressure, but there was no association between mild or marked levels of enhanced light reflex and mortality (all-cause or vascular).

Diet and the retinal microvasculature findings

After excluding persons with cataract, we found that increasing consumption of vitamin C (OR for 3rd tertile 1.83, 95% confidence interval, CI, 0.91 to 3.67, $p_{trend}=0.04$) was associated with the markedly enhanced retinal arteriolar wall reflex, and participants with greater than the median level intake of all micronutrients had a 4-fold higher odds of having the marked arteriolar wall light reflex. There were no associations with dietary intake and the mildly enhanced arteriolar light reflex.

Previous evidence suggests that narrower retinal arteriolar diameter and wider venular diameter is associated with increased risk of cardiovascular diseases. In this analysis, we found that increasing frequency of consuming any or oily fish was associated with both wider arteriolar diameter (p=0.002) and narrower venular diameter (p=0.02), both healthier states of these small vessels, after adjusting for cardiovascular risk factors, diet, inflammatory factors and socioeconomic status.

We found that increasing glycemic index (hazard ratio, HR, 1.91, 95% CI, 1.01-3.47, highest vs. lowest tertile) and decreasing cereal fibre (HR, 2.13, 95% CI, 1.19-3.80, lowest vs. highest tertile) predicted greater risk of stroke death, after adjustment for stroke risk factors. Increasing glycemic index and decreasing cereal fibre were also associated with retinal venular calibre widening ($p_{trend}<0.01$) and importantly, adjustment for retinal venular calibre attenuated stroke death risk associated with high glycemic index by 50%.

Diet and AMD findings

Multivariate models demonstrated that higher mean dietary glycemic index was associated with an increased 10-year risk of early AMD (relative risk, RR, 1.77, 95% confidence interval, CI, 1.13–2.78, comparing 4th to 1st quartile, $p_{trend}=0.03$). Conversely, greater consumption of cereal fibre (RR 0.68, 95% CI 0.44-1.04, $p_{trend}=0.05$) was associated with a reduced risk of incident early AMD. Similarly, in multivariate models increasing quartile of egg consumption was associated with a reduced risk of late AMD (relative risk, RR 0.29, 95% confidence interval CI, 0.12–0.70, comparing 4th to 1st quartile, $p_{trend}=0.01$).

Diet and cataract findings

There was a 40% reduction in the risk of developing nuclear and PSC cataract among participants in the highest compared to the lowest tertile of omega-3 fatty acids (p_{trend}=0.01 and p_{trend}=0.06, respectively) consumption. Higher consumption of long chain omega-3 fatty acids was associated with a 35% reduction in 10-year risk of nuclear cataract. Frequent consumption of fish (RR 0.75, 95% CI 0.54-1.04, p_{trend}=0.05) and nuts (RR 0.54, 95%CI 0.36-0.81, p_{trend}=0.003) was also associated with a reduced risk of nuclear cataract.

Additionally, we found that participants with higher consumption of vitamin B12 was associated with a progressively reduced risk of nuclear cataract (p_{trend}=0.0006). Those in the highest quintile of dietary riboflavin (p_{trend}=0.05) and calcium (p_{trend}=0.003) consumption were also associated with a reduced risk of PSC cataract. Investigating food sources of protein showed that consumption of legumes was also associated with a reduced risk of PSC cataract (5^{th} quintile: 40% risk reduction p_{trend}=0.04).

Diet and visual impairment findings

A Healthy Eating Index (HEI) score was calculated for each participant, based on a modified version of the Healthy Eating Index for Australians (HEIFA Greater HEI score (representing better diet and lifestyle) was associated with a 30% reduction in the relative risk of developing visual impairment (4^{th} quartile vs. 1^{st}: relative risk, RR, 0.60, 95% confidence interval, CI, 0.40 - 0.89, p_{trend}=0.016). There was a significant age interaction (p = 0.03): among persons ≥70 years there a significant reduction in the risk of visual impairment was evident but this association not seen in persons aged< 70.

Conclusions: Dietary factors may influence microvascular structural change or remodelling, which in turn may explain a substantial proportion of the longitudinal association between dietary factors and vascular disease. A diet with a low mean glycemic index may be of benefit in the primary prevention of ARMD and egg consumption may reduce the risk of developing late ARMD. A lower risk of cataract is associated with having a diet high in legumes, omega-3 fatty acids and fish. Finally, our result that persons who have consumed a healthy diet and engaged in a healthy lifestyle were less likely to have visual impairment over the long-term, provides additional support to the overall consistency of our project findings about the influence of one's diet to one's health.

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And to my 'pyari' Nani who I lost in the last stages of this thesis, I dedicate this work to you.

Candidate's Contribution

1. The candidate conceived of the primary hypothesis for the thesis topic. For chapters 5, 6, 7, 9, 10, 11 the candidate devised the primary hypothesis, for chapters 3, 4, 8, 12 the candidate, in conjunction with supervisors Jie Jin Wang and Paul Mitchell, devised the primary hypothesis.
2. The candidate graded fundus photographs for the entire baseline Blue Mountains cohort (3654 x 2) for the enhanced retinal arteriolar reflex sign and entered grading data and cleaned the data for this sign. For the Blue Mountains 3 data the candidate graded 70% of the fundus photographs for presence of the focal retinal microvascular signs. For side-by-side grading of the three follow-up examinations that involved grading the BMES 1, 2 and 3 photos for each participant (both eyes), the candidate graded 40% of the fundus photographs for the presence of retinal microvascular signs.
3. The candidate assisted Dr. Jie Jin Wang in the side-by-side grading of AMD lesions for the Blue Mountains Study 10 year follow-up examinations.
4. The candidate also collected data on participants in an affiliated study concerning the age-related eye diseases of cataract and AMD named the "Cataract Surgery and Age-related Macular Degeneration Study."
5. The candidate completed statistical analysis for all the chapters of the thesis, except the last chapter which was completed by Elena Rochtchina, senior statistician with the Centre for Vision Research, Westmead Millennium Institute, Sydney. The candidate was advised in many important statistical areas by Dr. Jie Jin Wang. The candidate also received valuable statistical advice from Dr George Burtulusky and Dr. Annette Kifley.

6. The candidate was responsible for literature review, data interpretation (in assistance with Dr. Jie Jin Wang for some chapters) and writing of all papers relating to this thesis where listed as first author, and substantially contributed to all others when not listed as first author.

Publications arising from this Thesis

1. **Kaushik S**, Tan AG, Mitchell P, Wang JJ. Prevalence and associations of enhanced retinal arteriolar light reflex: a new look at an old sign. Ophthalmology 2007:114(1);113-120

2. **Kaushik S**, Kifley A, Mitchell P, Wang JJ. Age, blood pressure and retinal vessel diameter: separate effects of blood pressure and age. Invest Ophth Vis Sci, 2007:48(2);557-61

3. **Kaushik S**, Wang JJ, Flood V, Liew G, Smith W, Mitchell P. Frequency of fish consumption, retinal microvascular signs and vascular mortality. Microcirculation 2008;15(1):27-32

4. **Kaushik S**, Wang JJ, Flood V, Barclay A, Wong TY, Brand-Miller J, Mitchell P. Glycemic index and the 10 year incidence of age-related macular degeneration. AJCN 2008:88;1104-1110

5. **Kaushik S**, Wang JJ, Flood V, Wong TY, Barclay A, Brand-Miller J, Mitchell P. Glycemic index, retinal microvascular signs and vascular mortality. Stroke 2009:40;206-212

6. **Kaushik S**, Wang JJ, Flood V, Liew G, Smith W, Mitchell P. Omega fatty acids and reduced incidence of nuclear cataract, an ageing biomarker, *submitted AJCN, 2010*

7. **Kaushik S,** Mitchell P, Flood V, Wang JJ. Proteins, micronutrients and food sources, and the long-term incidence of cataract, *submitted Archives of Ophthalmology, 2010*

8. **Kaushik S,** Mitchell P, Flood V, Wang JJ. Is Diet related to the Prominent Retinal Arteriolar Light Reflex Sign? *Submitted Ophthalmology, 2010*

9. **Kaushik S,** Wang JJ, Flood V, Wong TY, Mitchell P. Eggs and Age-Related Macular Degeneration. *Completed, awaiting submission.*

10. **Kaushik S,** Wang JJ, Flood V, Allman-Farinelli M, Bauman A, Wong TY, Mitchell P. Healthy diet and lifestyle protects against long-term visual impairment. *Completed, awaiting submission.*

Publications pertaining to, but not included in this Thesis

1. **Kaushik S**, Wang JJ, Mitchell P. Retinal vessel diameter. Ophthalmology 2006:113(5);886-887

2. Liew G, **Kaushik S**, Rochtchina E, Tan AG, Mitchell P, Wang JJ. Retinal vessel signs and 10-year incident age-related maculopathy: the Blue Mountains Eye Study. Ophthalmology 2006:113(9);1481-7

3. Tan JSL, Wang JJ, Flood V, **Kaushik S**, Barclay A, Brand-Miller J, Mitchell P. Carbohydrate nutrition, glycemic index and the 10-year incidence of cataract. Am J Clin Nutr 2007;86(5):1502-1508

4. Kifley A, Liew G, Wang JJ, **Kaushik S**, Smith W, Wong TY, Mitchell P. Long term effects of smoking on retinal microvascular calibre. A J Epidemiol, 2007;166(11):1288-1297

5. **Kaushik S**, Wang JJ, Mitchell P. Sleep apnea and falls in the elderly. J Am Geriatr Soc, 2007:55(7);1149-1150

6. Liew G, Mitchell P, Wong TY, Lindley RI, Cheung N, **Kaushik S**, Wang JJ. Retinal microvascular signs and cognitive impairment. J Am Geriatr Soc, 2009:57;1892-1896

7. Wang JJ, Cugati S, Fong CS, de Loryn T, Rochtchina E, **Kaushik S**, Tan J, Arnold J, Smith W, Mitchell P. Co-morbidities and short-term risk of age-related macular degeneration in an Australian cataract surgical cohort. *Submitted Archives of Ophthalmology, 2010*

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Abbreviations

APC	Antioxidants in Prevention of Cataracts Study
AREDS	Age Related Eye Disease Study
AMD	Age related macular degeneration
AV nicking	arterio-venous nicking
ATBC	Alpha-tocopherol beta-carotene study
BLSA	Baltimore Longitudinal Study of Aging
BMES	Blue Mountains Eye Study
BDES	Beaver Dam Eye Study
CAREDS	Carotenoids in Age-Related Eye Disease Study
CHS	Cardiovascular Health Study
CRAE	central retinal arteriolar equivalent
CRVE	central retinal venular equivalent
DHA	docosahexanoic acid
EES	European Eye Study
EDDCCS	Eye Disease Case-Control Study
EPA	eicosapentaenoic acid
FDA	Food and Drug Authority, United States
FFQ	Food frequency questionnaire
GI	Glycemic index
HEI	Health Eating Index
HPFS	Health Professionals Follow-up Study
ICD	International Classification of Diseases

ISH	International Society for Hypertension
JHPC	Japanese Public Health Center-based Study
LAST	Lutein Antioxidant Supplementation Trial
LOCS	Lens Opacities Case-Control Study
LSC	Longitudinal Study of Cataract
NDI	Australian National Death Index
NHANES	National Health and Nutrition and Examination Survey
NHS	Nurses Health Study
NVP	Nutrition and Vision Project
MVIP	Melbourne Visual Impairment Project
PHS	Physician's Health Study
WARMGS	Wisconsin Age-Related Maculopathy Grading System
WHI	Women's Health Initiative
WHO	World Health Organization
WHS	Women's Health Study
VECAT	Vitamin E, Cataract and Age-related Maculopathy Trial

Part 1

Literature Review and Aims of Thesis

Chapter 1: Diet, age-related macular degeneration, cataract, retinal microvasculature and visual impairment

High-quality diets are one of the principal environmental factors that have the potential to prolong the period of healthy life by preventing age-related diseases.[1] This is critical both in the West and in low and middle income countries, where age-related and chronic disease prevalence is rising.[2] Cardiovascular diseases, cerebrovascular diseases, cancer, diabetes and age-related eye diseases such as age-related macular degeneration (AMD) and cataract represent some of these diseases, the former four of which are the leading cause of mortality in the world, accounting for 60% of all deaths.[2] In fact, 80% of these deaths occur in developing countries.[2] Apart from poor diet, tobacco use and physical inactivity are the main other risk factors for these age-related diseases.[2]

The microvasculature represents the bulk of the circulatory system and dysfunction of this system is thought to precede important age-related vascular diseases such as hypertension.[3] Recent studies have demonstrated that changes to the retinal microvasculature can predict stroke, hypertension, coronary heart disease and diabetes.[4-17] Retinal microvasculature imaging presents an opportunity to investigate the dietary effects on this component of the circulation.

The age-related eye diseases of cataract and AMD are in the top 3 causes of blindness worldwide.[18] They represent a large burden of illness both in high and low income countries. Prevention represents the bulk of current research, but newer treatments for AMD are also becoming available. Dietary intervention is one of the few modifiable factors for ocular disease. This section provides a comprehensive review of nutritional studies in relation to ocular diseases. Although some consensus has been established for antioxidant supplements and AMD,[19-21] for other dietary components and for age-related cataract there is little consistent evidence. The Blue Mountains Eye Study is a cohort study that provides a unique opportunity to

study nutritional factors given its longitudinal follow-up period and the availability of a validated method of ascertaining dietary intakes and eye disease.

Retinal Microvascular Signs

The retinal microvasculature is amenable to high resolution photography and thus to in-depth analysis. This is a burgeoning new field of study. The retinal microvascular signs are divided into two groups.[22;23] The first group are 'focal' signs which incorporate retinopathy lesions, focal arteriolar narrowing, arterio-venous nicking and the enhanced retinal arteriolar light reflex. The continuous signs refer to retinal arteriolar and venular calibre.

Retinopathy lesions are graded based on the Early Treatment of Diabetic Retinopathy Study (ETDRS) and include microaneurysms, haemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates and new retinal vessels.[24] Focal arteriolar narrowing refers to a constricted area of arteriole that is 2/3 or less than the width of the arteriole. Arterio-venous nicking refers to the constriction of a venule on either side of an arteriole, at an arterio-venous crossing point. The enhanced retinal arteriolar light reflex refers to the white band on the surface of retinal arterioles which is presumed to be a reflex of light. The width of this band determines the reflex severity.[25] Finally, the overall calibre of retinal arterioles and veins is measured quantitatively using a computer program that assess the calibre of vessels 0.5 to 1 disc diameter around the optic disc.[23;26-29] This is summarised as the 'central retinal arteriolar equivalent' (CRAE) and the 'central retinal venular equivalent' (CRVE).

AMD

AMD is a disorder in older persons, typically bilateral, affecting the macula region of the retina, where there is a progressive loss of central vision from degenerative and/or neovascular processes. The macula is located temporal to the optic nerve, between the two temporal arcades (see Figure 1.1). The macula is a small area in relation to the entire retinal area, but is responsible for most of the useful vision and a large percentage of the visual field (10%).[30] AMD is divided into two phases, early AMD which is comprised of drusen, hypopigmentations or hyperpigmentations.[31] This may or may not develop into late AMD which comprises two forms, the 'dry' form which is atrophy or thinning of the retina, where choroidal vessels become visible, and the 'wet' form where neovascularisation occurs.[31]. The most common classification system for AMD used in interventional trials and observational studies is from the Age Related Eye Disease Study[32] Smoking, age and family history are the strongest risk factors for development of AMD.[30;31]

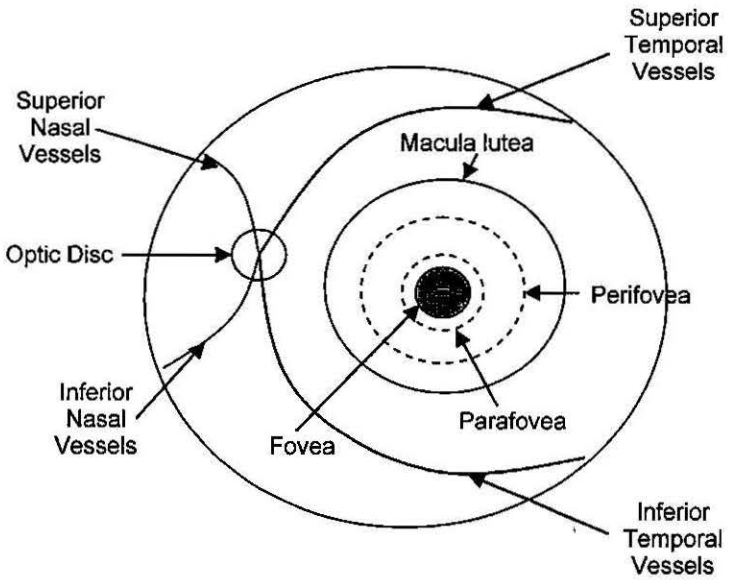

Figure 1.1. Schematic drawing of the posterior part of the retina, including the macula lutea (and its parts) and the optic disc.

Cataract

Cataract is any opacity of the human lens (see Figure 1.2). As the lens ages, new layers of fibres are added to the lens nucleus which is compressed and becomes more dense.[33;34] No fibres are lost with aging and new fibres are added to the outside of the lens, or the cortex of the lens.[33] Nuclear cataract is opacification of the central part of the lens, whilst cortical cataract is opacification of the outer layer of the lens. Posterior subcapsular cataract, the third most common type of cataract, is opacification of the very posterior part of the lens, just beneath the lens capsule. Typically age, trauma, steroid use and diabetes are important risk factors for the development of cataract.[33]

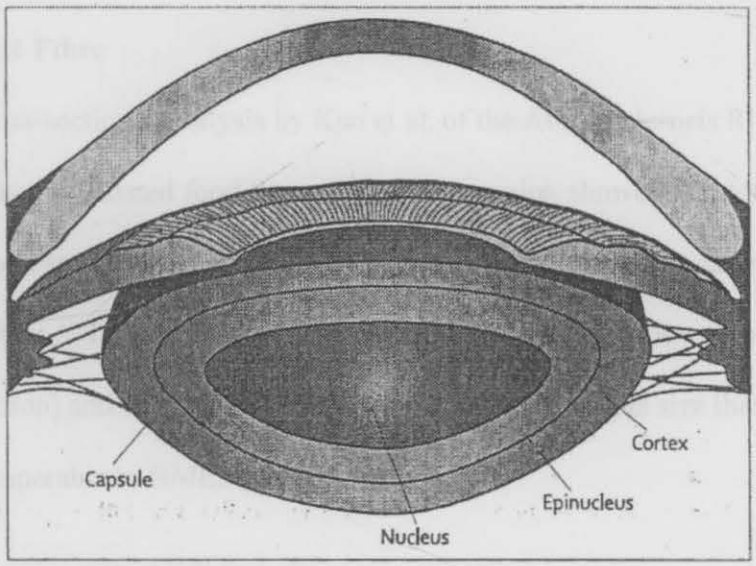

Figure 1.2. Schematic illustration of human lens in its position in the anterior chamber of the eye.

The parts of the lens, nucleus, epinucleus, cortex and capsule are labelled.[33]

1.1 Diet and Retinal Microvessels

Few studies have examined the area of diet and microvessels, with only one study,[35] discussed in the following section, published around the same time as the two chapters of this thesis.

Study of the retinal blood vessels through fundus photographs provides a unique way to examine the effects of dietary factors on the microcirculation. This is critical since disease of this component of the body's vasculature plays an important preliminary role in the development of lifestyle diseases such as coronary heart disease and stroke.[3;36] Analysis of the effects of diet on the microcirculation might suggest mechanisms by which certain nutritional components may adversely or positively affect the microvessels and thus produce or ameliorate these lifestyle diseases. Such in situ examination of the microvessels, within such a large cohort, as the BMES, allows for increased power and wider scope of research to explore new hypotheses that may not be available within the context of lab-based research.

1.1.1 Fibre

Cross-sectional analysis by Kan et al, of the Atherosclerosis Risk in Communities Study (USA) using a validated food frequency questionnaire, showed that total dietary fibre and fibre from cereal sources was associated with wider retinal arteriolar calibre and narrower retinal venular calibre.[35] This study used similar, validated grading protocols as the BMES (see Methods section) and was population-based with a large sample size that selected participants that were comparable to BMES participants.

Kan et al demonstrated results that were independent of various dietary factors, including total energy intake, omega-3 fatty acids, glycemic index, carotenoids, folate, vitamins B6, B12, C and E, and other sources of fibre.[35] Participants in the highest quintile of total fibre intake had arteriolar calibre that was 1.05μm larger and venular calibre that was 1.11μm smaller.[35] Although Kan highlighted previous relationships between arteriolar calibre and cardiovascular disease and suggested that fibre consumption might thus be beneficial to aspects of

cardiovascular pathogenesis, they did not statistically examine whether stroke or myocardial infarction risk was partly explained by retinal microvascular calibre. Hence their hypothesis remained untested. Kan et al[35] do however provide evidence that diet is associated with changes to the microvasculature, and thus, by extrapolation, suggest a mechanism through which dietary changes produce vascular disease. Fibre, in particular, has been shown to be beneficial in reducing risk of cardiovascular disease[37] although the mechanism is unclear. The mechanism is thought to be either through reduction of blood pressure[38;39] or dyslipidaemia,[40] improvement of endothelial function,[41] or reduction of inflammation.[42-44] Studies are needed that may provide better indication of the intermediary mechanism between fibre consumption and vascular disease.

1.1.2 Vitamin B12, folate and homocysteine

Although dietary intake of vitamin B12 and folate have not been examined, two studies have analysed serum levels of these inter-related factors in relation to retinal microvessel calibre.[45;46] Hyperhomocysteinaemia has been shown to be a strong, independent biomarker for coronary heart disease, peripheral vascular disease and venous thrombosis in multiple studies.[47-49] Homocysteine is thought to result in vascular disease by the induction of specific genes mediating cell proliferation, DNA hypomethylation, vascular inflammation and endothelial dysfunction.[48;50]

Without examination of systemic disorders, the study using BMES data by Gopinath et al,[45] showed that within the full cohort there was no association between serum homocysteinaemia, folate and vitamin B12 and retinal vascular calibre. Subgroup analysis however suggested an adverse effect in men (1.53μm narrowing of retinal arterioles per standard deviation, SD,

increase in plasma homocysteine) rather than women, which the authors suggest may be due to the differential vascular risk factor profile between men and women. The HOORN study,[46] which had comparable participants and grading methodology, also showed a relationship between homocysteinaemia and retinal arteriolar narrowing (3.8μm difference for every SD increase in plasma homocysteine), independent of age, gender, glucose tolerance status, cardiovascular risk factors and metabolic components of homocysteine. As a comparison, hypertension is related to small changes in arteriolar narrowing (each 10mmHg increase in systolic blood pressure has been associated with a 1.1 micron reduction in arteriolar diameter).[51]

Hyperhomocysteinaemia has been shown to be associated with microvascular disease.[52-54] Thus these studies provide further evidence of the effect of homocysteine on the microcirculation. Whether these associations actually mediate the vascular disease risk can be well investigated by population-based studies and provide an enhanced understanding of the pathogenesis of vascular disease.

1.1.3 Glycemic Index and Omega-3 fatty acids

No studies have specifically examined the relationship between retinal microvessel signs and glycemic index or omega-3 fatty acids.

1.2 Diet and Age-related Macular Degeneration

Dietary relationships with AMD have been extensively investigated by both observational and interventional studies. This is not surprising given that curative treatments that restore vision are lacking, that AMD is a significant cause of blindness, especially in Western populations, and that the prevalence will rise with ageing of the population.[55]

This section provides a comprehensive review of studies relating to dietary factors and AMD, in particular those studies concerning antioxidants, including lutein and zeaxanthin consumption.

1.2.1 Antioxidants, including lutein and zeaxanthin

Of all the dietary parameters, nutritional antioxidants have been examined the most, in the theory that oxidative damage is intimately involved with AMD. The idea that the retina is susceptible to oxidative damage is supported by many factors including its high concentration of polyunsaturated fatty acids, extensive light exposure and its high oxygen content.[56;57] There are many endogenous anti-oxidant agents. Endogenous anti-oxidants comprise enzymes such as superoxide dismutase, high molecular weight proteins such as albumin and low molecular weight anti-oxidants such as vitamin E.[58] The most common antioxidant agents that can be nutritionally administered are Vitamin E, C and carotenoids.[58]

Interventional trials in the area of antioxidant supplements and AMD are greater in number than in other nutritional or eye disease areas, (Table 1.1). In general, there is no trial evidence currently to support the use of antioxidants for the primary prevention of AMD.[19;59] However, in persons with signs of early to intermediate AMD, there is evidence to suggest that there is a benefit of supplementation of either zinc, or an antioxidant combination, for delaying progression to late AMD.[20] The latter result is largely provided by the AREDS interventional trial which involved 3640 participants in centres across the United States of America.[21] The AREDS trial reported an almost 30% reduced risk of late AMD (relative risk, RR, 0.72, 99% confidence interval, CI, 0.52-0.98) in participants taking an antioxidant combination with zinc.[21] Extrapolation of the AREDS data suggests that even after discontinuation of the supplement, there was benefit in the antioxidant supplemented group after 10 years (unpublished data,

American Academy of Ophthalmology meeting 2007) .This trial, however, did sample from retinal speciality clinics and was conducted in a predominantly Caucasian population. Other interventional trials were relatively under-powered to detect significant differences between treatment groups.[21;60-69]

There are further caveats to these findings. Firstly, adverse effects of long-term supplementation at the high levels reported by these trials are unknown. The length of time required to take the supplements is unknown, although as suggested above, there was a sustained benefit of treatment after discontinuation of the supplement. Secondly, many AREDS participants were already on a multivitamin supplement when the trial commenced. Whether these results, in such a highly nourished population, translate to other populations is unknown. Lastly, it would be of interest to know whether dietary modification through whole foods can provide similar benefits, given that they may be less likely to produce adverse effects and may result in systemic benefits.

In this regard many observational studies have provided evidence regarding the utility of fruits and vegetables or breads and cereals in risk reduction for AMD. These have been comprehensively summarised in Table 1.1. However, in general, cross-sectional, case-control and cohort studies have not demonstrated a consistent association between food sources, dietary estimation or supplement use of antioxidants and AMD. Furthermore, a meta-analysis of prospective cohort studies did not suggest a benefit for antioxidants in the primary prevention of AMD.[59] In this regard, further evidence, especially through interventional trials, would be helpful.

Table 1.1 also summarises the results of studies examining the carotenoids: lutein and zeaxanthin, lycopene, beta-carotene and alpha-carotene.

Lutein and zeaxanthin are 2 of 600 carotenoids.[70] All dietary carotenoids ultimately come from plants, but can be ingested from animal products such as egg yolk and milk.[70]There are around 30-50 carotenoids in the diet, 20 are measurable in serum, with the five main serum carotenoids being lycopene, beta-carotene, alpha-carotene, lutein and zeaxanthin.[70] Only lutein and zeaxanthin (macula pigments) are present in the retina,[70] most particularly in the macula lutea, and then decreasing in amount with increasing distance from the fovea. Their distribution in the retina, in addition to the localisation of late AMD and it relationship with lower macula pigment density, is thought to be evidence of the protective effects of lutein and zeaxanthin.[70;71] It is postulated that the main function of these carotenoids is in protecting the macula against photo-oxidative effects and in the filtration of blue-light.[70;71]

Only one interventional trial the Lutein Antioxidant Supplementation Trial (LAST) has examined oral supplementation of lutein (without zeaxanthin) alone or in combination with an antioxidant supplement in comparison with placebo, and examined clinically relevant end-points.[68] This trial was conducted in a small population, 90 male participants, over a period of 12 months and demonstrated that lutein alone improved macula pigment optical density, visual acuity by a mean of 5.4 letters, contrast sensitivity and was associated with reduced AMD progression, although there was low power for this latter finding.[68] In general, observational studies demonstrate a more consistent inverse relationship of lutein and zeaxanthin and neovascular AMD,[72-74] with no evidence of benefit for early AMD.[75-81]

A recent Food and Drug Authority (FDA) review of this area suggested that there was limited evidence for the benefit of lutein and zeaxanthin in the prevention or treatment of AMD.[82] The review cited many trials that have been conducted in this area, however suggested that these trials lacked power, control groups, assessment of clinically relevant end-points (such as development of AMD or visual acuity rather than macular pigment optical density) and may have used treatments that were not exclusive for lutein and zeaxanthin content.[82] It also suggested that observational studies do suffer from issues regarding recall of exact foods eaten, with differences in cooking methods altering food content, and similarly concern regarding whole foods comprising many dietary components in addition to the factor under study.[82] Thus although this area is promising, large interventional trials will be important to clarify any beneficial effects of carotenoids for AMD. Such a trial is in the process of being conducted by the AREDS team (AREDS II), and should provide results in the next few years.[83] Nevertheless, although specific components of food may be important, whole foods, through their provision of a multitude of perhaps more bioavailable nutrients, could provide more effective dietary interventions.

1.2.2 Fats

This area of dietary consumption has also received extensive study. The hypothesis underlying associations between lipid intake and AMD was first postulated using the theory that AMD and cardiovascular diseases may share a common pathogenesis or risk factors.[84] In fact, animal models show that high-fat diets can produce precursors of AMD lesions in the context of nonphototoxic light levels.[85;86] Studies examining the range of dietary fat intake from total fat to polyunsaturated fatty acids and cholesterol have demonstrated variable findings. However, in the case of omega-3 fatty acids, there has been a more consistent protective relationship.[87]

Omega-3 fatty acids, in particular long chain omega-3 fatty acids (docosahexanoic acid, DHA, eicosapentaenoic acid, EPA) are thought to have many cellular actions within the retina that are anti-angiogenic and neuroprotective, and may be able to ameliorate the effects of certain environmental exposures.[87] DHA is a major phospholipid of the retinal photoreceptor outer segment membrane and is also present in retinal microvessels.[87-89] The body does not have the capacity to meet tissue needs for long chain omega-3 fatty acids, and tissue levels are altered by dietary modification.[87] Omega-3 fatty acids are required for visual processing capacity, cell signalling, retinal cell gene expression, differentiation and survival of the sensory neuroretina.[87] In the vascular retina, they affect a multitude of molecules including eicosanoids, angiogenic factors, matrix metalloproteinases, reactive oxygen species.[87] In this way, they are postulated to protect against ischemic, phototoxic, oxidative, inflammatory and ageing pathology of the vascular and sensory retina which are thought to be important in AMD pathogenesis.[31;87]

Studies do not show a consistent association between total dietary fat, saturated fat, monounsaturated fat and polyunsaturated fat.[90-103] Most cross-sectional studies[90;93;94;97;99;102] with the exception of Heuberger et al[98] suggest an adverse relationship between either early or late AMD and dietary fat intake. Two cross-sectional studies, BDES[99] and the Cardiovascular Health Study (CHS)[102] suggested a relationship between a high intake of saturated fat, monounsaturated fat and cholesterol and early AMD, although in the CHS this was insignificant when analysing with all other variables. The CAREDS, studying a population from the Women's Health Initiative Study, suggested that total dietary and saturated fat was associated with intermediate AMD, but only in younger women.[97] The cross-sectional analysis of the BMES population[90] showed an adverse relationship between dietary cholesterol and late AMD. The Melbourne Collaborative Cohort Study[94] did not find any relationship with total or

saturated dietary fat, but rather with trans-unsaturated fatty acids and higher risk of late AMD. In contrast, the NHANES III, found no significant association between AMD and dietary fat, but of course 24-hour recall dietary assessment with single eye fundus photography was used in the study, which could have led to recall bias or under ascertainment of cases.[98]

Prospective studies are less consistent; with the BMES 5-year population-based incident analysis suggesting a 70% increased risk of early AMD in those with high consumption of total dietary fat.[101] However this finding could not be replicated in the 10-year incident BMES data which found no association between total dietary fat and incident AMD.[100] Cho et al[92] examined older subsamples of the Nurses' Health Study and Health Professional Follow-Up Study (HPFS) and reported a marginally significant increase in incident AMD. This study was limited by the ascertainment of AMD, which was by self-report, confirmed by medical record review. In a small hospital-based sample followed for 4.6 years, Seddon et al found an increased risk of progression to late AMD in persons with high total, animal, saturated, monounsaturated, polyunsaturated and trans-unsaturated fat consumption.[95] Based on these prospective studies, there is mixed evidence for the relationship between dietary fat and AMD, although prospective data from the BDES, Rotterdam Eye Study and other studies would be of considerable use.

There is increasing evidence for dietary long chain omega-3 fatty acids providing protection against AMD.[104] Case-control subsamples from the AREDS trial, as well as longitudinal analysis of the same cohort convincingly demonstrates a reduced risk of neovascular AMD and geographic atrophy in persons with higher dietary consumption of DHA, EPA and fish.[105-108] This finding was also demonstrated in prevalence, 5-year follow-up and 10-year follow-up data from the BMES.[90;100;101] Prevalence results showed an inverse association with late AMD and

fish consumption, 5-year follow-up analysis of the cohort demonstrated a reduced risk with early AMD, and latest 10-year data showed a relationship with long chain omega-3 fatty acids and reduced risk of early AMD.[90;100;101] The hospital-based study referred to earlier[95] also showed similar findings of a protective association between fish consumption and progression to late AMD. Finally a recent large European population (the Eureye Study) reporting an inverse association of oily fish, EPA and DHA consumption with neovascular AMD.[109]

Systematic reviews on the primary prevention[104;110] or delay in the progression of AMD,[111] in relation to omega-3 fatty acids and fish consumption have generally concluded that there is insufficient evidence from the literature to support advice about supplementation, though both authors recognise the strong suggestion of benefit. The current AREDS extension trial is also testing the role of omega-3 fatty acid supplementation of the risk of AMD progression, in addition to lutein and zéaxanthin.[83]

1.2.3 Glycemic index

This is a newer area of interest, but the few studies on this subject, from different populations, have been consistent in their findings. The glycemic index of a diet is used to characterise the postprandial glycemic response from consumption of a carbohydrate meal.[112-114] It is theorised that higher glycemic index diets allow greater amounts of glucose to enter cells, thereby increasing the potential for oxidative stress, formation of glycation products and subsequent inflammatory and angiogenic responses that lead to AMD.[115;116]

Diets high in glycemic index were found to be cross-sectionally related to retinal pigmentary abnormalities[115], large drusen (both components of early AMD) and severity of AMD[117]

independent of carbohydrate intake. Prospective analysis of the AREDS cohort demonstrated a greater risk of a diet high in glycemic index, resulting in AMD progression in persons with early AMD,[118]. Moreover, this study suggested that the risk was greater if the degree of baseline AMD was more advanced.[118] Further analysis by the same research team suggested that there was a lower risk of progression of AMD in persons who took the AREDS supplement but were in the lowest strata for dietary glycemic index or DHA and EPA consumption.[119] Recent prospective analysis of the Melbourne Visual Impairment Project (MVIP) showed that total carbohydrate intake was associated with incident cortical cataract (RR, 3.19, 95% CI, 1.10-9.27), however authors did not assess glycemic index and there was a large percentage missing data in the study. [120] This carbohydrate result is not consistent with studies described above.[115;117;118]

1.3 Diet and Cataract

In 2003, it was projected that over the next 10 years, the number of persons with cataract would grow by two thirds, from 1.67 million to 2.74 million by 2021.[121] The burden this places on cataract surgical services will be immense, and thus identification of modifiable risk factors, such as diet, that may reduce the prevalence of cataract is of great importance. This scenario is even more relevant to developing nations where cataract surgical services are already stretched.

1.3.1 Antioxidants, including lutein and zeaxanthin

The area of dietary antioxidants and cataract is one that has been extensively studied over the past two decades. Table 1.2 summarises the myriad cross-sectional, case-control, cohort and interventional studies in this area. As can be surmised from the Table, interventional studies have not really found a benefit of dietary or supplement use of antioxidants and incidence or

progression of cataract.[122-131] In contrast, prospective studies have almost all shown a protective relationship with some form of antioxidant, either vitamin E, C, especially lutein and zeaxanthin and their food sources.[132-145] Many of these prospective studies however, have used subsamples of cohort studies intended for other purposes,[132;133;135-137;139-141;144;145] and used self-report as the basis of ascertainment of cases or clinical examination.[132-137;142;144;145]

The biological basis for treatment of cataracts with antioxidants is suggested by extensive lab-based research emphasising the progressive, age-related increase in lens protein oxidation as a mechanism for cataractogenesis.[146] Hammond et al demonstrated a relationship between macular pigment optical density, which is thought to be a marker for lutein and zeaxanthin concentration in the macula, and lens optical density, which suggests an important function for lutein and zeaxanthin is protecting against cataract formation.[147;148] The antioxidant functions of lutein and zeaxanthin may be the mechanism of this protection.[149] Unfortunately at this time, there is insufficient evidence from the literature supporting dietary antioxidant advice for primary prevention or prevention of progression of cataract.

1.3.2 Protein

Many studies,[122;132;139;140;150-162] including an interventional study in China,[122] have investigated the relationship between dietary protein consumption or micronutrients related to protein, and the occurrence of cataract. Some of these studies[122;150;151;158;159] but not all[155;162] have shown that a diet with low levels of protein consumption is associated with an increased risk of cataract. Dietary riboflavin has consistently been identified as an important nutrient related to the occurrence of cataract in clinical studies[122;134;139;140;152;154;155;161] and biochemical studies that assessed riboflavin associated enzyme activity in serum.[151;152;160]

The association of cataract with protein or protein-related micronutrients may represent a reduced risk of disease in well-nourished persons living a healthy lifestyle rather than any benefit of protein per se. The Linxian study was the only interventional trial that demonstrated a benefit of riboflavin and niacin supplement use (or a multivitamin supplement) in relation to nuclear cataract.[122] The possibility that this resulted from an effect on a poorly nourished population cannot be ruled out.

1.3.3 Fats

In contrast to AMD, few studies have examined the relationship between dietary fat consumption and cataract. The two prospective studies data are consistent, with the 16 year follow-up analyses of the full cohort of the Nurses Health Study, using self-reported cataract surgery as the outcome, demonstrated that women with high omega-3 fatty acid intakes or those who consumed fish more than three times per week, had a 12% lower risk of cataract extraction.[163] These findings are supported by BMES 5-year incident analysis, reporting a 40% reduction in nuclear cataract risk associated with higher intake of long chain omega-3 fatty acids.[159]

In contrast, smaller subsamples of the Nurses Health Study, [164] with shorter follow-up periods have suggested that alpha-linolenic acid (ALA), an omega-3 fatty acid, was associated with *greater* change in lens nuclear opacification. This was observed in cross-sectional analysis of the same subsample as well.[165]

Omega-3 fatty acids may reduce the formation of cataract through their anti-oxidant, anti-inflammatory or anti-ageing properties.[166;167] Given that polyunsaturated fatty acids are present

in lens cell membranes,[168;169] and omega-3 fatty acids play an important role in improvement of lens membrane fluidity, enhancement of lens membrane function[170;171] may also be a mechanism by which these compounds reduce cataractogenesis, since changes in the lipid composition of the lens membrane can promote protein oxidation.[34;168;169]

1.3.4 Glycemic index

Similar to glycemic index and AMD, only a few epidemiological studies have investigated the association between glycemic index and cataract. The findings to date have not consistently demonstrated an association with cataract or cataract extraction. The Age-Related Eye Disease Study (AREDS) found that higher GI predicted nuclear, but not cortical cataract [172]. Neither the Nutrition and Vision Project (NVP), which examined cortical and nuclear opacities [173], nor the Nurses' Health Study (NHS) combined with the Health Professionals Follow-up Study (HPFS), which examined cataract extractions and nuclear and posterior subcapsular cataract subtypes, found an association with GI [174].

There is a putative role for glycemic index in cataractogenesis. High glycemic index diets produce a greater insulin response and a higher level of postprandial glycaemia.[112-114] It is well-established that diabetes is risk factor for cataract [175-178] and the severity of serum hyperglycaemia (HbA1c) is associated with an increased risk of nuclear and cortical cataract among people with diabetes .[175] The mechanism may be that the sustained exposure of lens proteins to elevated glucose causes accumulation of polyol and glycation, which may then produce oxidation, cross-linking, aggregation, and precipitation of lens proteins. [116;179-181]. It is observed that glucose can be taken up from the aqueous humour (filtered from the blood) by the lens.[173] Inflammatory effects of higher glycemic index diets could also be responsible for

cataractogenesis in addition to their oxidative capabilities. In this regard, higher glycemic index diets have been associated with greater plasma concentrations of the inflammatory marker C-reactive protein (CRP).[182]

Table 1.1. Cross-sectional, case-control, cohort and interventional studies examining dietary antioxidants and supplements in relation to age-related macular degeneration

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Cross-sectional studies					
Goldberg, 1988 (NHANES)[183]	-Probability sample of the 1971/1972 National Health and Nutrition Examination Survey -24-hour recall of foods high in Vitamin A and C	3082	Outcome was clinical fundus exam: neovascular AMD *and* pigmentary abnormalities	Fruit and vegetables high in vitamin A and C by frequency of intake	-Fruits and vegetables high in vitamin A, not C, inversely related to prevalence of any AMD -Confounding factors not collected such as smoking
West, 1994 (BLSA)[184]	-Sample from the Baltimore Longitudinal Study of Aging -Questionnaire on supplement use	976	Fundus photographs graded based on Chesapeake Bay study[185]: drusen, hyperpigmentation, geographic atrophy and neovascular AMD	Vitamin supplement use	-No protective effect of supplement use with AMD (any or severe?) -Fewer persons with late AMD
Mares-Perlman, 1996 (BDES)[76]	-Retrospective analysis of Beaver Dam Eye Study cohort: 10 year period -Food frequency questionnaire	1968	Fundus photographs graded using the WARMGS scheme[186] for early and late AMD	Foods +/- supplements of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin C, E, and zinc	-No relationship between antioxidants and AMD -Retrospective design for cohort study asking for past diets – high risk of recall bias

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Smith, 1999 (BMES)[187]	-Blue Mountains Study cohort -Food frequency questionnaire, assessing supplement use	2900	Fundus photographs graded using the WARMGS scheme[186] for early AMD	Food sources, dietary intake and supplement use of: carotene, retinol, vitamin c and zinc	-No association between dietary or supplement use of antioxidants and early or late AMD -Insufficient power for late AMD cases
Mares-Perlman, 2001(NHANES)[73]	-Probability sample of the 1971/1972 National Health and Nutrition Examination Survey -24-hour recall	8596	Fundus photographs graded using the WARMGS scheme[186] for early and late AMD	Lutein and zeaxanthin	-No relationship between lutein and zeaxanthin and early or late AMD -High level of non-participation (53%)
Kuzniarz, 2002 (BMES)[188]	-Blue Mountains Study cohort -Food frequency questionnaire, assessing supplement use	2873	Fundus photographs graded using the WARMGS scheme[186] for early and late AMD	*Supplements* only of: multivitamin, vitamin A, C, E, B12, niacin, thiamine, riboflavin, pyridoxine, folate, zinc	-No relationship between supplement use and early AMD -Carotene use associated with increased prevalence of late AMD - Multiple statistical comparisons -No correction for dietary vitamin intake -Insufficient cases for late AMD assessment

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Vu, 2005 (MVIP)[189]	-Cross-section of Melbourne Visual Impairment project -Food frequency questionnaire	2594	Fundus photographs graded using the WARMGS scheme[186] and International classification[190] for early and late AMD	Lutein and zeaxanthin, in conjunction with linoleic acid	-Inverse relationship between lutein and zeaxanthin in those with low linolenic acid intake and no supplement users -High loss to follow-up and 24% missing data
Moeller, 2006 (CAREDS)[191]	-Cohort from the Women's Health Initiative study recruited into CAREDS: 6 year prevalence -Women only -Food frequency questionnaire	1787	Fundus photographs graded using the AREDS scheme[32] for *intermediate* AMD (combinations of large drusen, extensive intermediate drusen, pigmentary abnormalities)	Lutein and zeaxanthin and fruits and vegetables	-No benefit in overall sample -Subgroup analyses suggested benefit of 'stable' lutein and zeaxanthin intake in women <75 years for intermediate AMD
Fletcher, 2008 (EES)[192]	-European Eye Study cohort -Food frequency questionnaire	4753	Fundus photographs graded based on the International classification system[190]	Dietary zinc (amongst other variables)	No association between dietary zinc and early or neovascular AMD, nor with adjustment for blue light exposure

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Case-control studies					
Seddon, 1994 (EDDCCS)[72]	-Clinic-based sample -Age- and sex-matched -Food frequency questionnaire, assessing supplement use	876	Neovascular AMD diagnosed by clinical examination, fundus photographs and fluorescein angiography	Retinol, alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamins A,C, E and supplement use and vegetable intake	-Carotenoids, spinach inversely associated with prevalence of neovascular AMD -No beneficial effect of supplement use -Given case-control design, high probability of association of diet with other healthy behaviours and recall bias
Snellen, 2002[74]	-Clinic-based sample -Age-matched -Food frequency questionnaire	138	Neovascular AMD based on clinical examination	Antioxidant 'score', lutein intake	-Antioxidant score unrelated to AMD -Lutein inversely related to neovascular AMD -Unmasked interviewers administered questionnaire -Small clinic sample

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
AREDS, 2007[81]	-Sample from the AREDS cohort -Age-matched -Food frequency questionnaire	4519	-Fundus photographs graded using the AREDS system[32] for presence of progression from intermediate AMD to late AMD	Lutein and zeaxanthin, beta-carotene, beta-cryptoxanthin, lycopene, vitamin C, E	-Lutein and zeaxanthin inversely associated with lower odds of late AMD and intermediate drusen -No association for other dietary variables -Clinic-based case-control but high proportion of controls were volunteers -High risk of recall bias
Cohort Studies					
Van den Lengenberg, 1998 (BDES)[77]	-Longitudinal analysis of the Beaver Dam cohort: 5 year follow-up -Food frequency questionnaire	1586	Fundus photographs graded using the WARMGS scheme[186] for early AMD only	Quintiles of alpha and beta-carotene, lutein and zeaxanthin, vitamin C, E, zinc	-Modest inverse association between carotenoids, vitamin e and large drusen; zinc and pigmentary abnormalities -Few late AMD cases
Christen, 1999 (PHS)[193]	-Cohort of the US Physicians Health Study: 12.5 person years of follow-up -Self-reported vitamin supplement use -No foods assessed	21120	Self-reported diagnosis of (any) AMD, confirmed by medical record review	Supplement vitamin C, E or multivitamin use	-No significant association between vitamin C, E and AMD -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Cho, 2001 (NHS and HPFS)[194]	-Nurses Health Study cohort and Health Professionals Follow-up Study cohort -10 years follow-up NHS; 8 years follow-up HPFS -Food frequency questionnaire, assessing supplement use	66 572 women 37 636 men	Self-reported diagnosis of early and late AMD, confirmed by medical record review	Zinc from diet and supplements	-No association between zinc and incident any, early or late AMD in men or women -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care
Flood, 2002 (BMES)[78]	-Blue Mountains Study cohort: 5 year follow-up -Food frequency questionnaire, assessing supplement use	2335	Fundus photographs graded using the WARMGS scheme[186] for early AMD	Diet and supplement use of: alpha and beta-carotene, beta-cryptoxanthin, retinol, lycopene, lutein and zeaxanthin, vitamin a, c and zinc	-Increased odds of early AMD with increasing vitamin C intake -No association between other dietary or supplement use of antioxidants and early AMD -Insufficient power to detect smaller differences
Cho, 2004 (NHS and HPFS)[75]	-Nurses Health Study cohort and Health Professionals Follow-up Study cohort -18 years follow-up NHS; 12 years follow-up HPFS -Food frequency questionnaire, assessing supplement use	77 572 women 40 866 men	Self-reported diagnosis of early and late AMD, confirmed by medical record review	Fruits and vegetables and diet and supplement use of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin A, C, and E	-No association between antioxidants or vegetables and incident any, early or late AMD in men or women -Inverse relationship between fruits and incident neovascular AMD -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
van Leeuwen, 2005[79]	-The Rotterdam Eye Study: mean follow-up of 8 years -Food frequency questionnaire administered by dietician	5836	Fundus photographs graded for early and late AMD based on the International classification system[190]	Dietary and supplement use of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin A, C, E, zinc and iron	-High dietary intake of beta-carotene, vitamins C, E and zinc were associated with reduced risk of any AMD
Arnarsson, 2006[195]	-Cohort of the Reykjavik Eye Study: follow-up of 5 years -Food frequency questionnaire	1045	Fundus photographs graded for early and late AMD based on the International classification system[190]	Tomatoes, red peppers and cucumbers	-Increased risk of AMD in those consuming low amounts of these vegetables -Low study power for late AMD
Tan, 2007 (BMES)[100]	-Blue Mountains Study cohort: 10 year follow-up -Food frequency questionnaire, assessing supplement use	2083	Fundus photographs graded using the WARMGS scheme[186] for early and late AMD	Dietary and supplement use of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin a, c, zinc and iron	-Highest decile of zinc less likely to develop early/any AMD -Highest tertile lutein and zeaxanthin reduced neovascular AMD -Higher alpha-, beta- carotene increased neovascular AMD -Low power and multiple tests were performed

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Cho, 2008 (NHS and HPFS)[80]	-Nurses Health Study cohort and Health Professionals Follow-up Study cohort -18 years follow-up NHS; 12 years follow-up HPFS -Food frequency questionnaire, assessing supplement use	71494 women 41564 men	Self-reported diagnosis of early and late AMD, confirmed by medical record review	Lutein and zeaxanthin (also stratified by vitamin C, E, BMI, smoking)	-No association between lutein and zeaxanthin and early or late AMD in the full cohort -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care
Interventional Studies					
Newsome, 1988[60]	-2 arms: placebo vs. zinc -Had early AMD with VA $\geq$ 6/24 -Follow-up between 12 and 24 months	151	-Visual acuity -Mean number of letters lost -Graded fundus photographs of increasing pigment, drusen and atrophy	Zinc	Zinc supplementation resulted in: -smaller decrease in mean visual acuity - no change in clinical features -less accumulation of drusen -No standardised grading protocol -Small sample size

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Holz, 1993[61]	-2 arms: placebo vs. zinc -Participants had drusen -Follow-up 12-24 months	58	Visual acuity, contrast sensitivity, dark adaptation, stereo fundus photographs and fluorescein angiograms graded for incidence of late AMD	Zinc	-Information available from abstract only -Modest benefit for zinc on incidence of late AMD
Kaiser, 1995[62]	-2 arms: Visaline vs. Placebo -All patients had atrophic AMD -Follow-up of 6 months	20	-Visual acuity -Contrast sensitivity -Retinal visual acuity -Colour vision	Visaline: beta-carotene, vitamin E, C	-No benefit of visaline against placebo in all measures of visual function -Short follow-up and very small sample size

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Stur, 1996[63]	-2 arms: placebo vs. zinc -Neovascular AMD in one eye, VA ≥ 6/12 in other eye -Follow-up 24 months	112	-Visual function: (visual acuity, Farnsworth-Munsell 100 score, retinal grating acuity, contrast sensitivity 3 cycles/degree, contrast sensitivity 18 cycles/degree) -Fundus photographs graded using the WARMGS scheme[186] for early AMD	Zinc	-No benefit of zinc on visual parameters -Insufficient power to detect development of neovascular AMD, but other high-risk morphological changes stable -Trial terminated early
Richer, 1996 [64;65]	-2-arms: placebo vs. antioxidant combination -Participants had drusen, RPE change on recruitment -Follow up of 18 months	71	-Visual function: snellen acuity, near vision M units with dual sided Bailey-Lovie chart, contrast sensitivity -Grading of fundus photographs	Combination of: beta carotene, vitamin E, vitamin C, citrus bioflavonoid, quercitin (bioflavonoid), bilberry extract (bioflavonoid), rutin (bioflavonoid), zinc, selenium, taurine, n-acetylcysteine, l-glutathione, vitamin B2	-Stabilisation of visual function parameters, but not fundus appearance

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Teikeri, 1998 (ATBC)[66]	-Sample from the ATBC (alpha-tocopherol beta carotene) study -Smoking males only -2×2 factorial AT BC AT + BC Placebo -Retrospective analysis: intervention given for 5-8 years	941	Fundus photographs graded at final follow-up only – occurrence of AMD -Graded by single ophthalmologist, no standardised protocol	Alpha-tocopherol (vitamin E) and beta-carotene	-No association between intake of AT and BC with any AMD -No standardised grading protocol, incomplete case ascertainment -Ophthalmic examination at follow-up only, so no true incidence data
AREDS, 2001[21]	-2 × 2 factorial -Placebo Antioxidant combination Zinc Zinc + antioxidant combination -4 groups which had extensive small drusen, intermediate drusen, large drusen, non-central geographic atrophy or pigmentary abnormalities and VA of ≥ 6/9 in one eye -Follow-up of 6.3 years	3640	-Fundus photographs graded using the AREDS system[32] for presence of progression from intermediate AMD to late AMD -Reduction in visual acuity ≥ 15 letters	Antioxidant combination - zinc, vitamin C and E, beta-carotene or zinc alone	-Reduction in development of advanced AMD with intake of antioxidants and zinc in *all* and *high-risk* participants -Reduction in moderate visual acuity loss with intake of antioxidants and zinc -Many participants were on vitamin supplements before trial start

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Taylor, 2002 (VECAT)[67]	-2-arms: placebo vs. vitamin E -Participants either had no or early AMD -Follow up of 4 years	1193	-Fundus photographs graded using the International classification system for incidence of early AMD or progression to late AMD -Visual acuity change	Vitamin E	-No benefit of vitamin E in the development or progression of early or late AMD -Low proportion of smokers -Under powered to detect progression of AMD
Richer, 2004 (LAST)[68]	-3-arms: placebo vs. lutein vs. lutein + antioxidant combination -All males -Participants had atrophic AMD -Follow-up of 12 months	90	-Fundus photographs graded by single ophthalmologist -Macular pigment optical density (MPOD) -Visual acuity -Contrast sensitivity	Lutein supplements or lutein + antioxidant supplement	-Lutein alone improved MPOD, visual acuity and contrast sensitivity -Lutein was associated with lack of progression of AMD -Small sample size -Non-standardised grading
Christen, 2007 (PHS)[69]	-2x2 factorial: placebo beta-carotene aspirin beta-carotene + aspirin -Part of the Physicians Health Study -All male -Follow-up of 12 years	22 071	Self-reported diagnosis of any and late AMD, confirmed by medical record review	Beta-carotene and aspirins	-No relationship between beta-carotene use and any or late AMD -Diagnosis relied on self-report, confirmed by ophthalmologist which may be inaccurate/under ascertainment

Table 1.1. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Newsome, 2008[196]	-2 arms: zinc vs. placebo -Participants had late AMD -6 months	80	Best corrected visual acuity, contrast sensitivity, light flash recovery time	Zinc monocysteine	-Participants in zinc group had significantly better visual acuity, contrast sensitivity and light flash recovery time
Christen, 2010 (WHS)[197]	-Cohort of the Women's Health study (WHS) -6-arms: beta-carotene vs. placebo, vitamin E vs. placebo, aspirin vs. placebo -Follow-up of 10 years	39 876	Self-reported diagnosis of AMD, confirmed by medical record review	Vitamin E	-No effect of vitamin E on development of any AMD -Subgroup analysis suggested benefit in smokers

Table 1.2. Cross-sectional, case-control, cohort and interventional studies examining dietary antioxidants and supplements in relation to age-related cataract

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Cross-sectional studies					
Mares-Perlman, 1994 (BDES)[156]	-Beaver Dam Eye Study cohort -Participants may or may not have had cataract at baseline -Retrospective cohort -Food frequency questionnaire, assessing supplement use – cross-sectional and 10 years prior	2152	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	*Supplements* only of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin C, E, riboflavin, thiamine, niacin, folate	-Supplement use of multivitamins 10 years in the past was associated with reduced odds of nuclear cataract, but increased odds of cortical cataract -Weaker cross-sectional relationships in same direction -Recall bias
Mares-Perlman, 1995 (BDES)[155]	-Beaver Dam Eye Study cohort – retrospective cohort -Only nuclear cataracts -Food frequency questionnaire - diet in the last year and 10 years before	3089	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	Foods +/- supplements of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin C, E, riboflavin, thiamine, niacin, folate	-Vitamin A, C, E, thiamine were associated with nuclear cataract in the full cohort -Association for niacin in men and folate in women -No relationship with food sources -Potential recall bias – dietary assessment was conducted after eye examination

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Jacques, 1997 (NHS)[199]	-Subsample of Nurses Health Study cohort -Food frequency questionnaire, biennial responses to same questionnaire amalgamated, assessing intake 13-15 years before baseline -Oversampling of those with high and low vitamin C intakes -Retrospective cohort	301	Single ophthalmologist performed slit –lamp examination using LOCS I[200]	Vitamin C	-Long-term consumption (>10 years) of vitamin C associated with lower prevalence of any opacity, in particular nuclear opacity -Low response rate for study -Possible bias related to vitamin C use change in relation to cataract diagnosis -Multiple measures of vitamin C supplement use over time
Cumming, 2000 (BMES)[158]	-Blue Mountains Study cohort -Food frequency questionnaire, assessing supplement use	2900	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	Protein, calcium, vitamin A, niacin, thiamine, riboflavin, iron, zinc Food sources	-Protein, vitamin A, thiamine, riboflavin associated with nuclear cataract - Multiple statistical comparisons

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Kuzniarz, 2001 (BMES)[154]	-Blue Mountains Study cohort -Food frequency questionnaire, assessing supplement use	2873	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	*Supplements* only of: multivitamin, vitamin A, B12, niacin, thiamine, riboflavin, pyridoxine, folate	-Multivitamins, thiamine and vitamin A inversely associated with nuclear or cortical cataract -Folate and vitamin B12 inversely associated with cortical cataract - Multiple statistical comparisons -No correction for dietary vitamin intake
Vu, 2006 (MVIP)[189]	-Cross-section of Melbourne Visual Impairment project -Food frequency questionnaire	2594	-Digital lens camera that produced Scheimpflug and retroillumination lens images used to grade incidence or progression of nuclear, cortical and posterior subcapsular cataract based on the Wilmer grading system[201]	Lutein and zeaxanthin	-Inverse relationship between lutein and zeaxanthin and prevalence of nuclear cataract -High loss to follow-up, with mean age included<mean age lost to follow-up -Low power for cortical, posterior subcapsular cataract

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Moeller, 2008 (CAREDS)[202]	-Sample from the Women's Health Initiative observational study -Nuclear cataract only -Food frequency questionnaire	1802	-Film slit-lamp camera and retroillumination camera lens photographs to nuclear cataract based on AREDS system[203]	Lutein and zeaxanthin	-High dietary lutein and zeaxanthin associated with reduced prevalence of nuclear cataract
Case-control studies					
Mohan, 1989[151]	-Hospital-based case control study -India	1990	Cataract status assessed by ophthalmologists on slit lamp and referred to study	Various variables, including nutrition: protein, vitamin A, C, E, thiamine, riboflavin, calcium	-No effect of antioxidants, protein used as a surrogate for other nutritional factors, associated with nuclear, posterior subcapsular and mixed cataract -Possible selection bias and potential misclassification of cases
Leske, 1991 (LOCS)[153]	-The Lens Opacities Case-Control Study -Clinic-based -Food frequency questionnaire	1380	Slit lamp examination and film slit-lamp camera and retroillumination camera lens photographs graded using LOCS I[200]	Beta-carotene, lutein, vitamin E, C, thiamine, riboflavin, niacin, iron	-Riboflavin, vitamin C, E, beta-carotene inversely associated with cortical, nuclear and mixed cataract -Iron, thiamine and niacin also associated with inverse relationship with cortical, nuclear and mixed cataract

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Italian-American Cataract Study group, 1991[162]	-Italian-American Cataract Study -Clinic-based study	1477	Cataract status assessed by ophthalmologists on slit lamp and referred to study using LOCS I system[200]	Vitamin A, C, iron, thiamine, riboflavin,	-No association between nutritional factors and any cataract -Possible selection bias and potential misclassification of cases
Robertson, 1991[204]	-Canada, clinic-based study -Age- and sex- matched -Interview conducted for supplement use assessment	350	Cataract status assessed by ophthalmologist on slit lamp and referred to study	Vitamin C, E	-Vitamin C and E supplementation was associated with a lower odds of any cataract
Jacques, 1991[205]	-Tertiary clinic-based -'Immature' cortical, nuclear or posterior subcapsular cataract -Food frequency questionnaire, with assessment of supplement intake	112	Cataract status assessed by ophthalmologists in eye clinic	Vitamin C, E, carotene, fruits and vegetable	-Increased odds of cortical and posterior subcapsular cataract with low vitamin C or fruits and vegetables intake -Low power of study, small sample

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Tavani, 1996[157]	-Italy, clinic-based -Cases admitted for cataract extraction; controls from same hospital -Food frequency questionnaire	913	Cataract extraction was the method of case selection	Beta-carotene, calcium, vitamin C, D, E, folate and food sources	-Calcium, folate, vitamin E were inversely associated with cataract extraction -High possibility of selection bias -High possibility of control misclassification as they did not have eye examination
Lyle, 1999 (BDES)[206]	-Cohort of Beaver Dam Eye Study: follow-up of 5 years -Subjects had no cataract at baseline -Food frequency questionnaire for baseline and in past (10 years before baseline)	4926	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	Alpha- and beta-carotene, lutein, vitamin E, C, lycopene, beta-cryptoxanthin and food sources	-Inverse association between lutein and spinach over 10 years ago with nuclear cataract -No association of vitamin C, E and nuclear cataract in full cohort. Some benefits in subgroup analyses that were not *a priori* -Recall bias
Jacques, 2001 (NHS)[161]	-Subsample of Nurses Health Study cohort -Nuclear cataracts only -Food frequency questionnaire, biennial responses to same questionnaire amalgamated over 13-15 years before baseline -Retrospective cohort	478	Film slit-lamp camera and retroillumination camera lens photographs graded using LOCS III[207]	Diet and supplement use of: vitamin C, E, alpha- and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, riboflavin, folate	-Reduction in nuclear cataract with vitamin C consumption from diet and supplements as well as an inverse association with duration of vitamin C use -Method of sub-sampling unknown

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Valero, 2002[208]	-Spain -Age- and sex-matched controls -Food frequency questionnaire	677	Clinical examination by two independent ophthalmologists based on the LOCS II system[209]	Vitamin C, E, alpha- and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin	-No relationship between dietary factors and cataract -Possible interviewer bias
Cohort Studies					
Hankinson, 1992 (NHS)[132]	-Nurses Health Study cohort; 8 years follow-up -Food frequency questionnaire, assessing supplement use, biennial responses to same questionnaire amalgamated over 12 years	50 828	Self-reported diagnosis of cataract extraction, confirmed by medical record review	Vitamin A, C, E, riboflavin, from diet and supplements and food sources	-Reduced cataract extraction with dietary (not supplement) consumption of vitamin A and greens like spinach -Vitamin C supplements also reduced the risk of cataract extraction -Under ascertainment of cases, as based solely on surgery
Seddon, 1994 (PHS)[133]	-Cohort of the US Physicians Health Study: 5 years of follow-up -Self-reported vitamin supplement use -No foods assessed	17 744	Self-reported diagnosis of cataract or cataract extraction, confirmed by medical record review	Supplement vitamin C, E or multivitamin use	- Reduced risk of cataract with multivitamin use -No significant association between vitamin C, E -No assessment of incident cataract -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Leske, 1998 (LSC)[134]	-The Longitudinal Study of Cataract: median follow-up 4.8 years -Food frequency questionnaire	764	Clinical examination by two independent ophthalmologists based on the LOCS III system[207] for nuclear cataract	Multivitamins and vitamin E	-Reduction of nuclear cataract with multivitamin and vitamin E intake
Brown, 1999 (HPFS)[135]	-Health Professionals Follow-up Study cohort: 8 years follow-up -Food frequency questionnaire, assessing supplement use	36 644	Self-reported diagnosis of cataract extraction, confirmed by medical record review	Alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin A, C, E, retinol	-Modest inverse relationship between lutein and zeaxanthin, broccoli, spinach and cataract extraction -No assessment of incident cataract -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care
Chasen-Taber, 1999 (NHS)[136]	-Nurses Health Study cohort; 12 years follow-up -Food frequency questionnaire, assessing supplement use	77 466	Self-reported diagnosis of cataract extraction, confirmed by medical record review	Diet and supplement use: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin A, retinol as well as food sources	-Modest inverse relationship between lutein and zeaxanthin, spinach, kale and cataract extraction -No assessment of 'incident' cases -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Chasen-Taber, 1999 (NHS)[137]	-Nurses Health Study cohort; 12 years follow-up -Food frequency questionnaire, assessing supplement use	47 152	Self-reported diagnosis of cataract extraction, confirmed by medical record review	Supplements only of vitamin A, C, E	-No overall association with full cohort, subgroup effect of vitamin C on reduced risk of cataract -Long term users of vitamin A had a reduced risk of cataract extraction -No assessment of 'incident' cases -Possible under ascertainment of cases, could be differential if persons were more likely to use vitamins and seek medical care
Mares-Perlman, 2000 (BDES)[138]	-Beaver Dam Eye Study cohort: 5 year follow-up -Food frequency questionnaire assessing supplement use in the last 5 years; a small subsample provided information about diet in the last year and 10 years before	3089	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	Foods +/- supplements of: alpha and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, vitamin C, E	-Multivitamin, vitamin C and E use for longer than 10 years was protective against nuclear and cortical cataract -Potential recall bias

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Taylor, 2002 (NHS)[139]	-Subsample of Nurses Health Study cohort -Cortical and posterior subcapsular cataracts only -Food frequency questionnaires; biennial responses to same questionnaire amalgamated over 12 years	603	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	Vitamin C, E, alpha- and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, riboflavin, folate	-No benefit in full cohort -Reduction in cortical cataract with vitamin C consumption in women <60 years -Multiple measures of diet was a strength
Jacques, 2005 (NHS)[140]	-Subsample of Nurses Health Study cohort: follow-up of 5 years -Nuclear cataract only -Subjects may or may not be free of cataract at baseline -Food frequency questionnaire, biennial responses to same questionnaire amalgamated over 13-15 years	408	Scheimpflug, digitalised lens photographs graded for nuclear cataract and change in nuclear cataract density based on REACT trial[210]	Vitamin C, E, alpha- and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin, riboflavin, thiamine, niacin, folate	-Long term use of vitamin E, riboflavin, thiamine associated with reduction in progression of any cataract -Differential loss of participants important potential for bias re: vitamin C results -Multiple statistical tests performed -Multiple measures of diet was a strength

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
AREDS, 2001[141]	-Centrum offered to persons starting AREDS study to standardise the usage of non-study supplements -Baseline lens status did not affect study eligibility -Follow-up of 6.3 years	4590	-Film slit-lamp camera and retroillumination camera lens photographs to grade incidence or progression of nuclear, cortical and posterior subcapsular cataract based on AREDS system[203]	Centrum: vitamin A, C, E, B1, B2, B6, B12, D, folic acid, niacinamide, biotin, pantothenic acid, calcium, phosphorus, iodine, iron, magnesium, copper, zinc	-Centrum reduced 'any' opacity progression and was protective for nuclear cataract incidence -High risk of selection bias, attempt at minimisation by use of 'propensity score' -High risk for uncontrolled confounding
Yoshida, 2007 (JPHC)[142]	-Cohort of the Japanese Public Health Center-based study: follow-up 5 years -Subjects may or may not have been free of cataract at baseline -Food frequency questionnaire	35 186	Self-reported diagnosis of cataract or cataract extraction	Vitamin C	-Vitamin C protective against the development or progression of cataract -Under ascertainment of cases

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Tan, 2008 (BMES)[143]	-Blue Mountains Study cohort: 10 year follow-up -Food frequency questionnaire, assessing supplement use	2464	Film slit-lamp camera and retroillumination camera lens photographs graded using Wisconsin Cataract Grading System[198]	Dietary and supplement use of: beta-carotene, vitamin A, C, E and zinc	-Highest intake of vitamin C protective against the development of nuclear cataract -Above median intake of vitamin C, E, beta-carotene and zinc was also associated with reduced risk of nuclear cataract, but this may be driven by vitamin C -Random camera error fewer cases of nuclear cataract -Multiple tests were performed
Christen, 2008 (WHS)[144]	-Cohort of the Women's Health study (WHS): follow-up 10 years -Food frequency questionnaire -Those with cataract diagnosis excluded at baseline	35 551	Self-reported diagnosis of cataract or cataract extraction, confirmed by medical record review	Vitamin C, E, alpha- and beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin and riboflavin Vegetable food sources	-Higher intake of lutein and zeaxanthin and vitamin E associated with a reduced risk of cataract -Purports to look at 'incident' cases, but no formal baseline eye exam, and for those defined as early cases insufficient follow-up period -Possible under ascertainment of cases

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Rautiainen, 2010[145]	-Sample from the Swedish Mammography Cohort: follow-up of 8.2 years -Women only -Women with cataract extraction before 1997 were excluded -Food frequency questionnaire	24 593	Cataract extraction self-report confirmed with linkage to cataract extraction register	Vitamin C supplements	-Vitamin C supplement use *increased risk* of cataract extraction -Those who where older, used HRT or corticosteroids had a higher risk -Under ascertainment of cases as only cataract extraction, without any eye examination
Interventional Studies					
Sperduto, 1993 (Linxian)[122]	-2 trials I multivitamin vs. placebo II 8 group fractional factorial design: retinol + zinc, riboflavin + niacin, vitamin C + molybdenum, selenium + vitamin A + vitamin E -Follow-up of 5-6 years	I – 2141 II - 3249	-Three ophthalmologists conducted clinical examination for cataract - Two examiners were using LOCS I,[200] one examiner LOCS III[207]	I multivitamin II retinol + zinc, riboflavin + niacin, vitamin C + molybdenum, selenium + vitamin A + vitamin E	-I Reduction in nuclear cataract for those persons receiving multivitamin supplement -II Reduction in nuclear cataract in those persons taking riboflavin and niacin; possible deleterious effect for posterior subcapsular cataract -End-of-trial eye examinations only -Serious confounding issues as no eye exam at start -Differences in grading between examiners

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Teikeri, 1998 (ATBC)[123]	-Case-control subsample from the ATBC (alpha-tocopherol beta carotene) study -Smoking males only -2×2 factorial AT BC AT + BC Placebo -Analysis at end of trial: intervention given for 5-8 years	425	Incident cataract extractions confirmed by hospital discharge register	Alpha-tocopherol (vitamin E) and beta-carotene	-No association between intake of AT and BC with cataract surgery -Ophthalmic examination at follow-up only, so no true incidence data; thus also risk of confounders affecting estimate -Under ascertainment of cases as based on cataract extraction
Teikeri, 1998 (ATBC)[124]	-Sample from the ATBC (alpha-tocopherol beta carotene) study -Smoking males only -2×2 factorial AT BC AT + BC Placebo - Analysis at end of trial: intervention given for 5-8 years	1828	Cataract graded using LOCS II/ system[209] from film slit-lamp camera and retroillumination camera lens photographs	Alpha-tocopherol (vitamin E) and beta-carotene	-No association between intake of AT and BC with cataract -Ophthalmic examination at follow-up only, so no true incidence data

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
AREDS, 2001[125]	-2 × 2 factorial -Placebo Antioxidant combination Zinc Zinc + antioxidant combination -Ocular eligibility criteria based on AMD (media sufficiently clear in study eye) -Follow-up of 7 years	4629	-Slit-lamp camera and retroillumination camera lens photographs to grade incidence or progression of nuclear, cortical and posterior subcapsular cataract based on AREDS system[203]	Antioxidant combination - zinc, vitamin C and E, beta-carotene or zinc alone	-No effect of antioxidant combination on cataract incidence or progression -This was true for 'any' group, specific cataract types or cataract surgery -Most patients already had some degree of cataract on entry -Many participants were on vitamin supplements before trial start
Chylack, 2002 (REACT) [126]	-2-arms: antioxidant combination vs. placebo -Follow-up of 2-4 years -Participants selected from a clinic setting, then randomised -Presence of 'minimal' cataract at baseline	297	-Scheimpflug slit images of lens, digital retroillumination lens photographs with image analysis to grade progression of cataract based on LOCS III system[207]	Antioxidant combination of: beta-carotene, vitamin C, E	-Antioxidant group had a small effect of reduced cataract progression compared to placebo -Large number of losses to follow-up but these did not differ greatly in important factors from the main sample

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Christen, 2003 (PHS)[127]	-2x2 factorial: aspirin: placebo vs. beta-carotene no aspirin: placebo vs. beta-carotene -Cohort of the Physicians Health Study -All male -Follow-up of 12 years	22 071	Self-reported diagnosis of cataract or cataract extraction, confirmed by medical record review	Beta-carotene	-No relationship between beta-carotene use and cataract or cataract extraction -Possible subgroup effect on smokers at baseline – lower risk -Diagnosis relied on self-report, confirmed by ophthalmologist which may be inaccurate -Possible under ascertainment of cases
Christen, 2004 (WHS)[128]	-Cohort of the Women's Health study (WHS) -6-arms: beta-carotene vs. placebo, vitamin E vs. placebo, aspirin vs. placebo -Follow-up of 2.1 years	39 876	Self-reported diagnosis of cataract or cataract extraction, confirmed by medical record review	Beta-carotene	-No effect of study combination on development of cataract or cataract extraction -Subgroup analysis suggested benefit in smokers -Trial terminated early due to concerns about safety of beta-carotene, thus few cataract events and combined with small follow-up time possible reduced power

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
McNeil, 2004 (VECAT)[129]	-2-arms: placebo vs. vitamin E -Participants may or may not have had cataract -Follow up of 4 years	1193	-Digital lens camera that produced Scheimpflug and retroillumination lens images used to grade incidence or progression of nuclear, cortical and posterior subcapsular cataract based on the Wilmer grading system[201]	Vitamin E	-No benefit of vitamin E in the development or progression of 3 types of cataract -Low proportion of smokers -Randomisation produced equal numbers between groups
Gritz, 2006 (APC)[130]	-Antioxidants in Prevention of Cataracts Study (APC) in Andhra Pradesh, India -2-arms: antioxidant combination vs. placebo -Follow-up: 5 years	798	Film slit-lamp camera and retroillumination camera lens photographs graded using LOCS III[207] for nuclear opacity change with time, cortical and posterior subcapsular opacities	Antioxidant combination: vitamin A, C, E	-Antioxidant supplementation not associated with cataract progression -Negative results in a population likely to have nutritional deficiency -Good power

Table 1.2. continued.

Study	Design	Sample size	Outcome	Dietary Factors	Results/Quality
Christen, 2008 (WHS)[131]	-Cohort of the Women's Health study (WHS) -6-arms: beta-carotene vs. placebo, vitamin E vs. placebo, aspirin vs. placebo -Those with cataract diagnosis excluded at baseline -Follow-up of 9.7 years	39 876	Self-reported diagnosis of cataract or cataract extraction, confirmed by medical record review	Vitamin E	-No effect of vitamin E on development of cataract or cataract extraction -Reliance on self-report with possible under ascertainment of cases

1.4 Objectives of this Thesis

The objectives of this thesis were to:

1. Provide a new methodology for assessment of retinal microvascular arteriolar and venular calibre that accounts for the variance in interindividual vessel size.
2. Provide descriptive epidemiology of a hitherto poorly described sign, the enhanced arteriolar light reflex.
3. Use the aforementioned data to assess the previously unexplored association of diet with microvascular changes, including the enhanced retinal arteriolar light reflex, in relationship to systemic vascular disease.
4. Assess the nutritional relationships between the previously poorly explored area of glycemic index and egg consumption in relation to age-related macular degeneration.
5. Assess dietary relationships with incident cataract.
6. Analyse the novel relationship of dietary patterns with *incident visual impairment*, a functional indicator of ocular disease.

Part 2

Methods

Chapter 2: Population, data collection procedures and ascertainment of disease

2.1 Sampling and Study Population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision and common eye diseases in residents aged 49 years and over of a defined area, west of Sydney, Australia. The study adhered to recommendations of the Helsinki Declaration and was approved by the Sydney West Area Human Ethics Committee. Written, informed consent was obtained from all participants.

The BMES was conducted in a single, geographically well-defined region; the urban townships of Katoomba, Leura and Medlow Bath (postcode 2780) and Wentworth Falls (postcode 2782). This approach was chosen to increase the potential response rate as community support could be easily mobilised and data collection might be easier. A door knock census of 38 Australian Bureau of Statistics (ABS) census collection districts (CCDs) in these two adjoining postcode areas (2780 and 2782) was conducted in 1991. Of 4433 eligible residents, 3654 aged 49 to 97 years, attended an eye exam (response rate 82.4%) in the first examination period of January 1992 to January 1994. Details of recruitment methods are described in detail elsewhere.[211;212]

Overall the BMES population was similar to the Australian population. The BMES 1 population was slightly older, with more participants aged in their 60s and 70s, but a lower proportion in their 80s. The BMES 1 population contained a larger proportion of people who were either born in Australia or were from the United Kingdom and Ireland. They were more likely to be of higher socioeconomic status, as indicated by a higher proportion of flat/home ownership, a higher proportion of qualifications after leaving school and a lower proportion of manual workers than the Australian population.

BMES 2 study followed-up participants from the original BMES 1 cohort, five years later, from 1997-1999. From the original 3654 who participated in BMES 1, 2334 attended eye examinations for BMES 2, representing 75% of survivors (n=3111); 534 (14.9%) people had died, 383 (10.5%) had moved and 394 (10.8%) refused to participate.

Before commencing the BMES 3, 10-year examinations during 2002 through 2004, 1103 (30.2%) participants died, 375 (10.3%) moved from the study area, and 224 (6.1%) refused to participate. There were 1952 baseline participants (53.4% of the original cohort, or 76.6% of survivors) who were re-examined at the 10-year examinations.

2.2 Dietary Data

Dietary data were collected using a 145-item self-administered food frequency questionnaire (FFQ), modified for the Australian diet and vernacular from an early Willet FFQ (1988)[213]. Prior to attending the eye examination, participants were sent this FFQ for completion. A nine-category frequency scale was used to indicate the usual frequency of consumption of food items during the past year, and included in portion size estimates. It was produced using larger font for easier reading by older, potentially visually impaired persons. The FFQ used in BMES 2 and 3 was identical to that used in BMES 1, with the exception that additional information was collected about brand and type of certain foods that contained fortified folate: fruit juice, breakfast cereal and bread, and about types of meat used. An allowance for season variation of fruits and vegetables was made during analyses of all examinations by weighting seasonal fruits and vegetables.

The FFQ was validated against three separate 4-day weighed food records, collected over the period of one year to account for seasonal variation, in a randomly selected sub-sample of the BMES cohort (n=79). Validation of the FFQ has been described in a previous report.[214] Briefly, the FFQ was found to be reliable in this population and to have reasonable concurrent validity compared with weighed food records collected over 1 year.[214] Correlation coefficients (r) comparing the FFQ and weighed food records were over 0.50 for all nutrients except protein (r=0.16), vitamin A (r=0.32) and iron(r=0.37). For fats, the FFQ was found to show moderate to good agreement for ranking individuals according to their fat intakes, yielding correlation coefficients between 0.4-0.7, and correctly classifying over 70 percent of people within one quintile for all types of fats.[214] Dietary supplement intake was assessed by questionnaire, which sought details about whether a supplement was used, and if so, the frequency, brand and strength. Supplement data were coded using an updated supplement database previously developed for use in BMES 1.[215] Each nutrient's supplement data for every participant reflects the addition of all supplements used by that participant.

Participants with more than 25 missing values (17%) in their FFQ were excluded from the final dataset. Participants with 13-25 missing values in the FFQ were checked for data entry errors, which were corrected were possible. If, after data entry, more than 12 FFQ questions remained blank or an entire page remained blank, these FFQs were excluded from the final data set. Participants with FFQ daily energy intakes of less than 2500kJ of greater than 18000kJ were excluded from the final dataset. Finally, nutrient data were screened for extreme values, inspecting values in the upper and lower 2% of the distribution to locate and correct and data entry errors and to check for plausibility.

Dietary intakes in this study were estimated using a purpose-built software package, "the FFQ masher".[216;217] The FFQ masher incorporated the Australian Tables of Food Composition database (Nuttab90 in BMES1, Nuttab95 in BMES2 and Nuttab in BMES3) and a fatty acid supplement database 1999.[218;219]

Glycemic index data were obtained from the Sydney. University Glycemic Index Research Service (SUGiRS) online database (www.glycemicindex.com) and published values.[220] In total, 88.9% of glycemic index values were obtained from published values and 11.1% were interpolated from similar food items. An overall glycemic index value for each participant's diet was calculated by summating the weighted glycemic index of individual foods in the diet, with the weighting proportional to the contribution of individual foods to total carbohydrate intake.

A total of 3267 participants attempted and returned the FFQ, of which 2895 were sufficiently complete and plausible for analysis (79.2% of the participants examined; 88.6% of those attempted to complete the FFQ). Non-participants were of a similar age and sex distribution, but had a lower self-reported prevalence of spectacle wearing and hypertension.[221] Participants without usable FFQ data were of a similar sex distribution, but were more likely to be older, current smokers, to have hypertension and to have a lower self-reported prevalence of spectacle wearing than those with usable FFQs.[221]

2.3 Interview and Examination Procedures

All procedures were conducted according to a standardised protocol. At the clinic visit, trained interviewers completed a comprehensive demographic and medical questionnaire for each participant. The questionnaire included detailed questions regarding past and current

medications, family and social history, and past medical or surgical diagnoses and a self-rating of global health and vision. Questions were also asked about driving history, smoking status, alcohol intake and exercise. Alcohol intake was classified by the number of standard alcohol drinks consumed (defined as 10 grams or 12.5 millilitres of alcohol), according to the Australian National Health & Medical Research Council classification[222]. Smoking status was defined from history as never smoked, ex-smoker and current smoker (which included those who had ceased smoking within the last 12 months). Education was assessed by recording the attainment of a trade certificate or higher qualification, using the ABS Classification of Occupations.[223] Details of visits to either ophthalmologists or optometrists were recorded and verified as only two full-time optometrists worked in the area and two part-time ophthalmologists.

The eye examinations and interview procedures were conducted in different rooms. At the baseline examination we measure participant's height, weight and blood pressure. Systolic and diastolic blood pressure (SBP and DBP) were measured once, from the first and fifth Korotkoff sounds, using a single mercury sphygmomanometer with appropriate adult cuff size, after seating the participants for at least 10 minutes. Mean arterial blood pressure was defined as 0.33×systolic blood pressure +0.67×diastolic blood pressure. Body mass index (BMI) was calculated as weight(kg) /height $(m)^2$. Diabetes was defined either from past history of diabetes and current diabetes treatment, or from fasting plasma glucose levels ≥7.0mmol/L at examination, using the World Health Organization diabetes classification.[224] We followed the 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) guidelines[225] to define severe hypertension as grade 2 or 3 i.e. a SBP ≥ 160mmHg or DBP ≥ 100mHg at examination. Fasting blood samples were taken from 3222 (88%) of the 3654

participants.[226] the Institute of Clinical Pathology and Medical Research at Westmead Hospital performed laboratory tests within four hours of blood collection.

2.3.1 Assessment of deaths

Deaths that occurred from baseline were confirmed by cross-matching demographic information of the 3654 participants with the Australian National Death Index (NDI) data using probabilistic record linkage.[227;228] The sensitivity and specificity of Australian NDI data has been estimated to be 93.7% and 100 % for all-cause deaths, and 92.5% and 89.6% for cardiovascular deaths [227;228]. Cause of death was collected from death certificates by NDI and defined using International Classification of Diseases Code (ICD) versions 9 and 10. Coronary heart disease (CHD) related deaths were defined according to the following codes from ICD-9 (3949, 4029, 4109, 4119, 4140, 4148, 4149, 4151, 4240, 4241, 4254, 4269, 4273, 4274, 4275, 4278, 4280, 4281, 4289, 4290, 4291, 4410, 4411, 4413, 4414, 4415, 4439) and ICD-10 (I059, I10, I132, I219, I249, I251, I255, I259, I269, I271, I350, I352, I358, I429, I469, I48, I500, I514, I515, I516, I709, I711). Stroke related deaths (thrombotic, hemorrhagic) included the following ICD-9 codes (4300-4389) and ICD-10 codes (I600-I699) when listed as any cause of death.

2.3.2 Measurement of visual acuity

Visual acuity was measured using a LogMAR chart (Vectorvision CSV-1000™ Vectorvision Inc, Dayton, Ohio) and read at 2.4 metres. The CSV-1000 has a fluorescent light source that retro-illuminated a translucent chart. A series of photocells monitor and calibrate the light level to 85 candelas per square metre ± 0.1 log unit, which has been verified. Three measurements of visual acuity were performed for each eye. Vision was initially assessed using current distance glasses if worn, then with a 1.2 mm pinhole aperture held over current distance glasses and

finally after subjective refraction. If no letters could be read at 2.4m, the chart was moved to 95cm (0.4 log units) closer to the participant. If no letters could be identified on the chart at 95cm, the vision was assessed as 'count fingers' at 0.5m, 'hand movements', 'perception of light' or 'no perception of light'.

Each eye was covered sequentially, and the participant was asked to read down the chart slowly, letter by letter. Each letter read correctly was recorded, and only one reading of each letter was allowed. When the subject had difficulty with a letter, he or she was encouraged to guess. There was also an illiterate HOTV test face and a hand-held prompt card.

Visual acuity was recorded as the number of letters read correctly, from 0 (<6/60) to 70 (6/3). The scores were adjusted according to the test distance. Participants who could read 54 letters or more (Snellen equivalent 6/6 or better) without spectacles were not tested for astigmatism or refractive error, which was considered insignificant in such cases and recorded as emmetropic. If participants could read 54 letters or more with current distance correction, then the readout from the lens analyser was entered as the refractive error.

If visual acuity with present glasses was less than 54 letters (6/7.5 or 20/25), the autorefractor correction was placed in a trial lens frame and a subjective refraction was performed using the Beaver Dam modification of the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol.[229]

Participants underwent pupillary dilatation with tropicamide 1% and phenlyephrine 10% and a fundus examination was conducted in all subjects, including slit lamp and retroillumination

photographs of the lens and stereoscopic photographs of the optic disc and retina. At the 5- and 10- year follow-up visits, participants were examined in approximately the same order as at baseline, using the same procedures and equipment.

2.4 Ocular Photography and Eye Disease Ascertainment Procedures

At each visit, 30 degree stereoscopic retinal photographs of the macula and optic disc, plus non-stereoscopic photographs of four other retinal fields (temporal, nasal, and both upper and lower vascular arcades), as described previously[211] using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkechen, Germany). Photographs of both eyes were obtained in 98%, or of at least one eye in 99% of participants at baseline and at BMES 2,[230] and of both eyes in 85% or of at least one eye in 87% of participants at the 10-year examinations.

2.4.1 Retinal microvascular signs grading

Trained, masked graders assessed 35mm retinal slides of both eyes taken at baseline for focal arteriolar narrowing, arterio-venous (AV) nicking and the enhanced retinal arteriolar light reflex.[22;231] Focal arteriolar narrowing, AV nicking (nicking) and presence of retinopathy lesions (microaneursyms, haemorrhages) were graded utilizing only arterioles located at least one-half of a disc diameter from the optic disc-margin and by comparison with standard photographs selected from the set developed for the Modified Airlie House Classification of Diabetic Retinopathy (photographic standards 2A, 8A, 10A and 14)[232] and the Wisconsin Age-Related Maculopathy Grading System (cases 54, 8, 58)[233]. Focal arteriolar narrowing was graded as absent/questionable (none/less severe than the standard) or present (equal to or more severe than the standard). AV nicking was graded as present when constriction was evident on both sides of a venule where crossed by an arteriole. AV nicking was graded as absent/questionable, mild, or

severe. The quadratic weighted kappa statistic for intra-grader reliability for detection of focal arteriolar narrowing and AV nicking was 0.80 and 0.87, respectively. The grading of non-diabetic retinopathy lesions has previously been reported.[234]

Retinal photographs were assessed by graders for presence of none, questionable, mild or marked enhancement of the retinal arteriolar light reflex.[25] Presence of mildly or markedly enhanced light reflex was graded from 35mm slides of both eyes using a light box (Kelvin rating approximately 6200 degrees) and Donaldson stereoscopic viewer with x5 magnification. Photographic standards were selected by a retinal specialist (Paul Mitchell) from the slide set of the Modified Airlie House Classification of Diabetic Retinopathy and BMES participant photographs. Mild enhancement of the retinal arteriolar light reflex was defined as the presence of a reflex with a sharp margin, one-third the width of the arteriolar vessel and consistently present (without interruption) over at least two-thirds the length of the arteriolar sector visible in the field. The width of the reflex was compared with that in the mildly enhanced retinal arteriolar light reflex photographic standard.[25] Marked enhancement of the retinal arteriolar light reflex was defined as presence of a reflex whose width was greater than one-third the arteriolar wall width, irrespective of its intensity but consistently present over at least two-thirds the length of the arteriolar sector visible in the field.[25] The grading of markedly enhanced arteriolar light reflex was required to be similar to or more obvious than the markedly enhanced retinal light reflex photographic standard. Any enhancement of the arteriolar light reflex thus included both mild and marked levels. Although methods used for grading other focal vascular abnormalities have excluded the zone within one half disc diameter circumference from the optic disc, grading for the presence of an enhanced light reflex did not exclude this zone due to the requirement to assess consistency of the reflex along the whole course of arterioles. Grades

were adjudicated by a senior researcher, and for equivocal or difficult cases, by a retinal specialist (Paul Mitchell). The intra-grader reliability was assessed, with simple kappa statistic of 0.75 and weighted kappa statistic of 0.84.[25]

A computer-assisted grading method with high reproducibility was used to measure retinal vessel calibre, developed by the University of Wisconsin-Madison.[22;28;233] In brief, digitised retinal images of one eye (mainly right eyes) of each participant were displayed and all vessels greater than 25μm in diameter and completely passing through the region between 0.5 to 1 disc diameter from the optic disc margin were measured. Average arteriolar and venular calibres were calculated using the Parr-Hubbard formula.[23;28;29] Intra- and inter-grader grading reliability of this method were assessed previously,[28] with quadratic weighted kappa 0.85 for arteriolar measurements and 0.90 for venular measurements for inter-grader reliability and between 0.80-0.93 for intra-grader reliability.

Retinopathy lesions in subjects without diabetes were also graded using the modified ETDRS classification of diabetic retinopathy,[235;236] as previously reported.[237] Retinopathy was recorded if any of the following lesions were present: (1) microaneurysms, (2) blot or flame shaped hemorrhages at least 0.5 disc diameter from the disc margin, (3) hard exudates or (4) cotton wool spots.

2.4.2 AMD grading

Details of the photographic grading for ARM lesions performed in this study have been previously reported[211] and closely follow the Wisconsin Age-Related Maculopathy Grading System.[186] All photographs taken at each examination had an initial masked grading. Assessments of inter- and intra-grader reliability showed good agreement.[211] Side-by-side

grading of the baseline and 5-year photographs[230] and of the baseline and 10-year photographs, was then performed for participants with any AMD lesions identified at either follow-up examination.

Early AMD was defined, in the absence of late AMD, as presence at the macula of either: (1) large (>125 μm diameter) indistinct soft (or reticular) drusen or (2) both large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or hypopigmentation)[190;211] Late AMD was defined to include either neovascular AMD or geographic atrophy (GA), the two late-stage lesions described in the International AMD classification.[190] All late AMD cases detected from each examination were adjudicated and confirmed by a retinal specialist (PM).

Incident early AMD was defined by new appearance of early AMD lesions at follow-up examinations.[238] Participants with either distinct soft drusen or retinal pigmentary abnormalities at the baseline examination, but not both, who went on to develop complementary lesions that together comprised early AMD were included as incident early AMD cases.[238] Incident indistinct soft drusen or incident retinal pigmentary abnormalities were defined similarly among persons without early or late AMD. Incident late AMD was defined by the new appearance at follow-up of neovascular AMD or GA.

2.4.3 Cataract grading

Slit-lamp lens photographs were taken from each eye using Ektachrome 200 color film (Kodak, Rochester, NY) and an SL-7E photograph slit-lamp camera (Topcon, Tokyo, Japan) to assess presence of nuclear cataract. Retroillumination lens photographs were taken using a CT-R

cataract camera (Neitz Instruments, Tokyo, Japan) to assess presence of cortical and posterior subcapsular (PSC) cataract.

The Wisconsin Cataract Grading System, first developed in 1990 for use in the Beaver Dam Eye Study, was used to perform masked grading of the lens photographs. This method is well described.[239;240] A 5-point scale was used to assess the presence and severity of nuclear cataract, by direct comparison of participant photographs with 4 standard slit-lamp photographs of lenses with increasing opacity. Nuclear cataract was defined as nuclear opacity worse than standard 3. The presence and severity of cortical cataract and PSC were graded from Neitz photographs using a circular grid divided into 8 equal wedges and a central circle. Graders estimated the percentage area involved by cataract in each of the 9 segments. The opacity percentage in each segment was then summated to give a score for the whole lens area. Cortical cataract was considered present when the opacity involved at least 5% of the lens area. PSC cataract was defined if any was present. Inter-grader reproducibility for baseline photographs was 0.79 for nuclear, 0.78 for cortical, and 0.65 for PSC cataract. The 10-year follow-up photographs were graded by one examiner for all 3 types of cataract, and the same examiner graded a random sample of baseline photographs to compare inter-grader reproducibility. This gave weighted kappa 0.52 for nuclear, 0.72 for cortical, and 0.79 for PSC cataract. All positive cortical and PSC cataract cases were also graded by another senior grader. Nuclear and PSC cataract cases detected at all 3 examinations were either graded or re-graded by a senior researcher (Jie Jin Wang) whose intra-grader reliability was 0.79 for both nuclear and PSC cataract. Adjudication was provided by an ophthalmologist (Paul Mitchell).

2.5 Statistical Analysis

Details of statistical analysis are described with each chapter, however all statistical analyses were performed using the Statistical Analysis System (version 9, SAS Institute, Cary, NC). In all cases dietary variables were adjusted for total energy intake using the Willett residual method.[241]

Part 3.1

The Retinal Microvasculature

Chapter 3: Age, blood pressure and retinal vessel diameter: separate effects of blood pressure and age

Abstract

Purpose: The association between age, blood pressure (BP) and retinal vessel change is widely reported, with inverse relationships between retinal arteriolar and venular diameter and increasing age and elevated BP. No previous studies have dissected the separate effects of age and BP on the diameter of retinal vessels.

Methods: Population-based, cross-sectional study comprising 3654 participants (82.4% response) aged 49+ years from Blue Mountains region, Australia. Retinal arteriolar and venular diameters were measured from digital retinal images, using a standardised method and were summarised as central retinal arteriolar (CRAE) and central retinal venular (CRVE) equivalents.

Results: After adjusting for venular diameter, regression plots and regression coefficients from linear models demonstrated an inverse relationship between arteriolar diameters and mean arterial BP (MABP) in all age groups, greatest for the <60 age group and progressively diminishing thereafter. Increasing age was associated with greater arteriolar narrowing (of lesser magnitude), for each MABP category (<100mmHg, 100-109, 109-129, >120) with the greatest effect seen for persons with MABP<100. There was evidence of interaction between age and blood pressure in their effects on arteriolar diameter (p=0.003). After adjusting for arteriolar diameter, age was inversely associated with venular diameter (the effect was progressively greater for persons with progressively higher MABP), and MABP was positively associated with venular diameter in subjects aged <80 years (interaction p=0.05).

Conclusion: These findings demonstrate the importance of elevated blood pressure to arteriolar narrowing, especially in those younger than 60 and that venules tend to widen rather than narrow with increasing blood pressure levels.

Introduction

The association between age, blood pressure and narrowing of retinal arteriolar and venular diameters has been demonstrated in several population-based cohort studies. In the Blue Mountains Eye Study[22], Beaver Dam Eye Study[242], Atherosclerosis Risk in Communities Study[243], Cardiovascular Health Study[244] and most recently the Rotterdam Eye Study[51], a strong inverse relationship between retinal arteriolar diameter and both increasing age and elevated blood pressure was shown after adjusting for multiple confounders. Certain studies also demonstrated an inverse relationship between age and retinal venular diameter and a much weaker inverse relationship between blood pressure and retinal venular diameter[27;51;242].

To our knowledge, no studies have evaluated the separate effects of age and blood pressure on retinal arteriolar or venular diameters, after considering the shared variance between arterioles and venules. This report explores the separate effects of age and mean arterial blood pressure (MABP) on retinal vessel diameter, after stratifying for both MABP and age, as well as adjusting for shared variance between the two vessel diameters, in the Blue Mountains Eye Study baseline population.

Methods

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision, common eye diseases and other health outcomes in an urban predominantly Caucasian population aged 49 years or older. The baseline study, conducted during 1992-4, examined 3654 eligible potential participants living in two postcode areas in the Blue Mountains, west of Sydney, Australia (82.4% response). This study was conducted in accordance with recommendations of the Declaration of Helsinki and was approved by the Western Sydney Area Health Service

Human Ethics Committee. Written, informed consent was obtained from all participants. Details of recruitment methods were previously described[211;237].

A standardised interview and examination was performed on all participants over the period 1992-94. Information on demographic variables was gathered from the questionnaire. Questions were asked regarding lifestyle factors, including smoking. Participants were asked for details of current and past smoking, including the type (manufactured or hand rolled cigarettes, cigars, or pipe tobacco), usual amount (current and past), time of commencement and, where applicable, time of quitting. Current smokers were defined as participants who smoked cigarettes, cigars or a pipe regularly, and had given up smoking for less than 12 months prior to the examinations, whereas past smokers were defined as participants who had ever smoked cigarettes, cigars or a pipe regularly, but had given up for at least 12 months prior to the examinations. A single measure of resting, seated systolic and diastolic blood pressure using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. MABP was defined as 0.33 x systolic blood pressure + 0.67 x diastolic blood pressure. Body-mass index was calculated as weight (kg)/height $(m)^2$. Fasting blood samples were taken and processed for serum glucose. Blood samples were analysed at the Institute of Clinical Pathology & Medical Research, Westmead Hospital.

Stereoscopic retinal photographs (30 degree) of the macula and other retinal fields of both eyes, using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany), were taken at baseline. Detailed grading methods have been described previously[22]. In brief, we used a computer-assisted method, developed by the University of Wisconsin-Madison[233], to measure the internal diameter of retinal arterioles and venules from all gradable digitized photographs which were

then summarised using formulas by Parr and Hubbard[27;28]. The formulas take into account branching patterns of arterioles and allow individual vessel diameters to be combined into the summary indices, central retinal arteriolar (CRAE) and central retinal venular (CRVE) equivalent, reflecting the mean arteriolar and venular diameters respectively, of that eye.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis System (V8.2, SAS Institute, Cary, NC). In order to assess the effect of blood pressure or age on retinal arteriolar or venular diameter, independent of the fellow vessel component, we used venule-adjusted CRAE and arteriole-adjusted CRVE, calculated using the residual method initially described by Willet,[245] which controls for the shared variance between the two diameter variables. Venule adjusted-arteriolar diameter was defined using linear regression with venular diameter as independent variable and arteriolar diameter as the dependent variable, obtaining the residuals, and adding them to an expected mean venular diameter, given the size of arterioles. Similarly, arteriole adjusted venular diameter was defined using regression of arteriolar diameter as the independent variable and venular diameter as the dependent variable, with the residuals from the regression added to the expected mean arteriolar diameter. This method has been widely used in nutritional epidemiological research. The resulting adjusted variables, arteriole-adjusted CRVE and venule-adjusted CRAE, can be considered to represent the non-shared variance of each vessel diameter, respectively.

Regression graphs were generated displaying the linear relationship between MABP and venule adjusted-CRAE or arteriole adjusted-CRVE by four age groups: <60 years, 60-69 years, 70-59 years and 80+ years. The slope of each line demonstrates the effect of MABP on arteriolar

diameter, and qualitatively the 'gap' between the age group lines demonstrates the effect of age. Mean venule adjusted-CRAE and arteriole adjusted-CRVE were obtained using ANCOVA, stratified by age and MABP groups. Linear trends and regression coefficients for the changes in mean venule adjusted-CRAE or mean arteriole adjusted-CRVE (dependent variable) associated with increasing age stratified by MABP category, or the changes associated with elevated BP stratified by age group, were obtained. In these linear regression models, we included age (per decade, in models for MABP subgroups) or MABP (per 10mmHg, in models for age subgroups), and gender, body mass index, smoking, and serum levels of glucose, as independent variables. Interactions between age and MABP were examined qualitatively and tested in the multiple linear regression models.

Results

Of the 3654 baseline participants, photographs of the right eye of 3346 were included in analyses, after excluding 308 participants without retinal photographs, those with poor photographic quality that precluded measurement, those with retinal diseases that confounded measurement of retinal vessel width, or subjects who had missing or incomplete blood pressure data. The latter included 40 persons. The average age of the sample was 65.5 years. The number of persons in age groups <60, 60-69, 70-79 and 80+ years was 984 (29.3%), 1239 (36.9%), 864 (25.8%) and 268 (8.0%), respectively. The MABP for corresponding age groups was 101.7, 104.0, 105.9 and 106.3 mmHg, respectively.

Figures 3.1.1 and 3.1.2 illustrate the relationship between venule-adjusted mean CRAE, arteriole-adjusted mean CRVE and MABP, stratified by four age groups. In Figure 3.1.1, each consecutively older age group has successively smaller mean arteriolar diameters when MABP

was within the normal range (<100 mmHg), and within each age group, there was arteriolar diameter narrowing with increasing levels of MABP. There is a greater effect of MABP on arteriolar diameter in the younger age groups, indicated by the greater slope of decline in the younger age groups compared with the oldest group. This is supported by the data presented in Table 3.1.1, which show mean adjusted-CRAE and regression coefficients stratified by age group and the category of MABP. The regression coefficient of –2.3 per decade of age in persons with MABP<100 represents a 2.3 micron reduction in mean venule adjusted-CRAE for each decade increase in age. There is a progressive reduction in the absolute value of regression coefficients for the effect of MABP on CRAE in consecutively older age groups, and smaller regression coefficients for the effect of increasing age on CRAE in the higher MABP categories compared to that seen for persons whose MABP was within the normal range. The linear trends for change in arteriolar diameter associated with increasing MABP were significant in the three younger age groups, and the trend for change in arteriolar diameter associated with increasing age was significant only in those who were normotensive (MABP<100 mmHg). The qualitative impression of interaction between age and MABP on arteriolar diameter taken from Figure 3.1.1 and Table 3.1.1 was verified statistically (p=0.003).

Figure 3.1.2 demonstrates that younger age groups have wider venular diameters than older age groups. Conversely, within each age group except the oldest group, there was a linear trend for venular widening associated with increasing MABP. Table 3.1.2, which shows mean arteriole adjusted-CRVE and regression coefficients, stratified by age group and MABP categories, demonstrates progressively larger negative regression coefficients for change in venular diameter associated with increasing age in consecutively higher MABP categories, and smaller positive regression coefficients for change in venular diameter with increasing MABP in the two

middle age groups compared to the youngest one. For the oldest age group, the regression coefficient for changes in CRVE associated with MABP was negative. The linear trends for reduction in venular diameter with increasing age were significant in the three higher MABP categories (Table 3.1.2). Interaction between age and MABP on venular diameter was of borderline statistical significance (p=0.05).

Table 3.1.3 and 3.1.4 show the unadjusted (crude) CRAE and CRVE values, stratified by age group and MABP. In consecutively older age groups, there was successively smaller arteriolar diameter, which reduced with increasing MABP (Table 3.1.3). In the case of venular diameter, both increasing BP and age were negatively associated with a decrease in venular diameter, and there was a greater decline associated with increasing age than that associated with increasing MABP (Table 3.1.4). The findings for unadjusted CRVE and BP (Table 3.1.4) are opposite to the positive association between arteriole adjusted-CRVE and BP (Table 3.1.2). Finally, results were essentially unchanged when systolic blood pressure was used, instead of MABP.

Discussion

In this study, we attempted to investigate the separate effects of age and blood pressure on retinal arteriolar and venular diameter while controlling for influence from the correlated fellow component. We found the following: 1) The effect of blood pressure on arteriolar diameter narrowing interacts with age and is more prominent in younger persons (<60), and this effect of blood pressure on arteriolar diameter narrowing diminishes with increasing age; 2) The effect of age on arteriolar diameter narrowing also depends on blood pressure levels, and is greatest in persons within the normal blood pressure range than in persons within higher MABP categories;

3) Elevated blood pressure is positively associated with retinal venular diameter after adjusting for shared variance with arterioles, i.e. the higher the blood pressure, the wider the mean venular diameter, for subjects up to age 80 years; and 4) The effect of age on venular diameter narrowing is progressively greater for persons within progressively higher levels of MABP. Given that older persons and those with elevated blood pressure have initially narrower arterioles, further arteriolar narrowing (at a slope similar to that seen in younger or normotensive persons) is conceivably less likely to occur. Previous studies have suggested that this might be due to arteriosclerosis causing stiffening of arteries in older persons, thus limiting the degree of vasoconstriction that can occur[242].

To our knowledge, previous studies have not accounted for the shared variance between arteriolar and venular diameters, when evaluating the association between either arteriolar or venular diameter and age or blood pressure. Biologically, persons with smaller arterioles are naturally more likely to have smaller venules, which may be explained by body size or a commonality of genetic determinants between venules and arterioles within the same individual. This explains the considerable correlation between arteriolar diameter, CRAE, and venular diameter, CRVE (correlation coefficient, r =0.59). The arteriolar-to-venular ratio (AVR) was initially used to account for this correlation, but was misinterpreted as representing changes in *arteriolar diameter*[51;233] *by assuming stable venular diameter. When including the correlated* fellow vessel component in multivariate models (CRVE when CRAE is the dependant variable, and vice versa), the variation of CRAE explained by the resultant model is more than doubled, from less than 20% to more than 40%. Using the residual method to adjust for the fellow vessel component, we are able to eliminate the shared variance and thus assess associations with each vessel type independently. The potential confounding effect from the correlated fellow vessel

component has been documented previously.[11;12] The adjusted vessel diameter variables would permit an assessment of the effects of age and blood pressure on vessel diameter changes that was independent of the effect from the fellow component, while accounting for factors shared by the two retinal vessel diameter components, including the effects of body size and genetic determination.

However, there are some concerns about using this method and interpreting the adjusted variables. The adjusted vessel diameter variables should not be considered equivalent to the unadjusted diameter variables. We may have over-adjusted for the shared factors, or there may be the possibility of masking or exaggerating the associations assessed. For example, it may be possible that the observed widening of arteriole adjusted-venular diameter with increasing blood pressure represents the relative difference from arterioles, which narrow with increasing blood pressure, rather than an absolute change in venular diameter itself. Although there are biologically plausible links between widening venular diameter and elevated BP as outlined in the next paragraph, the interpretation of these findings should be undertaken with these possibilities borne in mind.

In this study, analyses using unadjusted arteriolar diameter showed that age and blood pressure had a similar magnitude and direction of effect on arteriolar diameter narrowing, whereas after adjustment for venular diameter, blood pressure had a greater effect on arteriolar diameter than age, though the effect direction remained the same. In contrast, analyses using unadjusted venular diameter showed that higher blood pressure was negatively associated with venular narrowing, whereas after adjusting for arteriolar diameter, blood pressure was positively associated with venular widening. This finding contrasts with previously reported cross-

sectional analytic results[27;242] of increasing blood pressure associated with narrower venular diameters, and a longitudinal association between venular narrowing and incident hypertension[246]. Nevertheless, we believe that our study findings using analyses that co-adjusted for vessel diameters avoid the potential confounding effect from the fellow vessel component[11;12] and are consistent with existing evidence. In the case of arteriolar diameter, it is consistent with known knowledge and the interaction found between blood pressure and age is biologically plausible. In the case of venular diameter it replicates our recently reported data on the association between venular widening and incident hypertension[11]. Such an association is also biologically plausible, as inflammatory processes and/or damage to vascular endothelium are likely contributors to the pathogenetic link between wider venular diameter and elevated blood pressure. Wider venular diameter has been shown associated with inflammatory factors such as C-reactive protein, interleukin-6, amyloid-A levels and markers of endothelial dysfunction[247], and accumulated evidence also points to an inflammatory pathogenesis for hypertension[248].

We have shown that higher blood pressure is associated with greater retinal arteriolar diameter decline compared with age across all age groups, albeit with a decreasing magnitude in older persons. This finding underscores the importance of regulating elevated blood pressure in all age groups. In addition, the implication of effect of increasing age on arteriolar diameter in persons who are normotensive deserves discussion. Current research is focused on identifying the differences between changes due to 'normal' aging and those due to pathologic processes associated with age, as well as identifying biomarkers that distinguish between age-dependant diseases and 'normal' aging. In terms of cardiovascular disease, age has traditionally been considered a dominant, non-modifiable risk factor. A review by Najjar et al showed that normal

ageing is accompanied by a number of structural and functional changes to the cardiovascular system[249]. Luminal dilatation of elastic arteries, increased arterial wall thickening and endothelial dysfunction are some of the changes described. The authors suggest that these changes observed in subjects with cardiovascular disease are similar to aging changes but occur at earlier ages. Our results showing a greater effect from aging on arteriolar diameter among normotensive subjects and a greater effect from elevated blood pressure in younger persons compared with older persons, suggest that hypertension accelerates aging-related arteriolar changes.

The current analyses demonstrated statistically significant narrowing of venules associated with age, independent of retinal arteriolar diameter. Though this has been reported in previous studies[27;242], the reason for this finding has not previously been explained. A pathological study has described age-related anatomical changes in the human renal veins[250]. It found that though the muscle fibre bundles atrophied with age, the elastic fibre bundles hypertrophied. Hypertrophy of elastic fibres may perhaps be a possible mechanism for the venular narrowing with age, found in our study. Conversely, age-related narrowing of retinal vessels, including arterioles and venules, demonstrated in our findings, may primarily be a vascular phenomenon or due to loss of retinal neurons associated with older age. Previous experimental studies have shown age-dependant reductions in ocular (both choroidal and retinal) blood flow[251;252]. Other studies have also shown that there is a loss of ganglion cells and their axons, photoreceptors and retinal pigment epithelial cells in the retina associated with age[253-255]. Such depletion of nerve fibre numbers could reduce ocular perfusion requirements, thus leading to the vessel narrowing observed. We are unable, however, to explain our findings of a stronger effect of age on venular diameter narrowing as blood pressure increases.

In summary, we used a novel method that accounts for correlation between arterioles and venules, and also for variation in vessel diameter associated with body size or genetic factors, to explore the relationship between retinal arterioles, venules and age or blood pressure. Our findings demonstrate an interaction between age and blood pressure on these small vessel diameters, and emphasize the importance of elevated blood pressure to arteriolar narrowing, especially in those younger than 60. We have also shown that venules widen rather than narrow with increased blood pressure. The effect of age on vessel diameter could be considered to be related not only to biological aging but also to age-related conditions other than elevated blood pressure. These findings, if confirmed in future studies, may have clinical implications in cardiovascular disease prevention and in studies of healthy aging.

Figure 3.1.1. Effect of mean arterial blood pressure (MABP) on venule-adjusted retinal arteriolar diameter (CRAE) by age group.

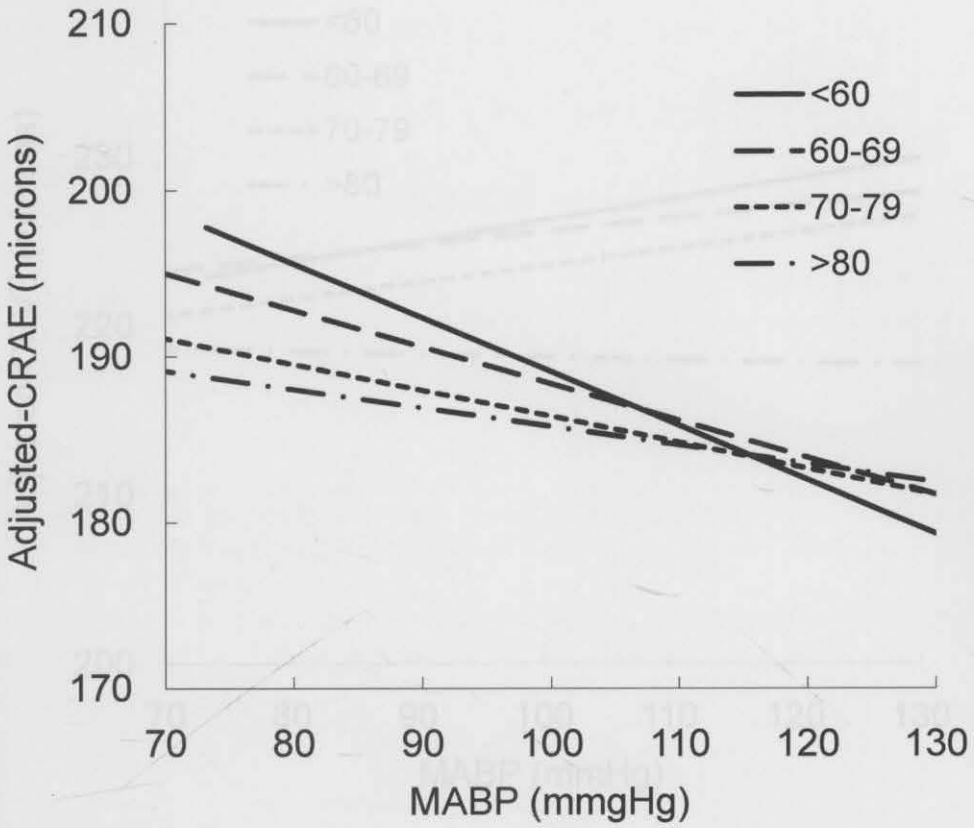

Figure 3.1.2. Effect of mean arterial blood pressure (MABP) on arteriole-adjusted retinal venular diameter (CRVE) by age group.

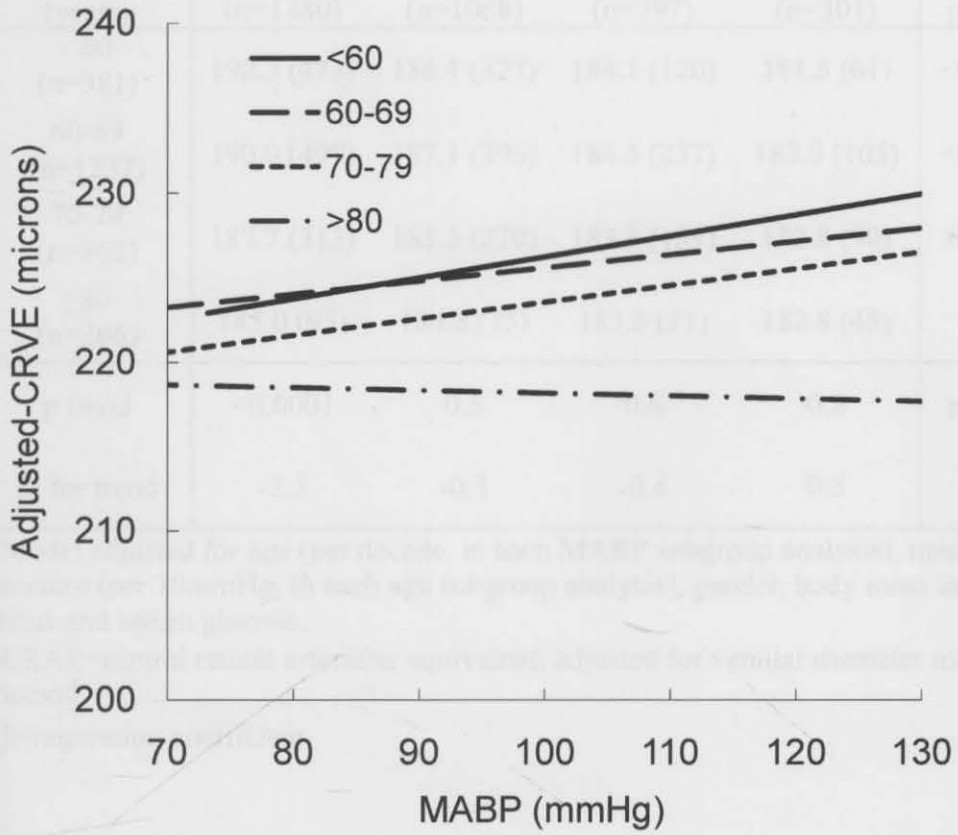

Table 3.1.1. Mean* venule adjusted-CRAE† (microns) stratified by age group and mean arterial blood pressure.

Age Group (years)	Mean arterial blood pressure (mmHg) <100 (n=1380)	100-109 (n=1068)	110-119 (n=597)	≥120 (n=301)	p trend	β‡ for trend
<60 (n=981)	192.3 (473)	186.1 (327)	184.1 (120)	181.5 (61)	<0.0001	-3.3
60-69 (n=1237)	190.0 (499)	187.1 (396)	184.5 (237)	183.9 (105)	<0.0001	-1.9
70-79 (n=862)	187.7 (313)	185.5 (270)	183.5 (189)	182.8 (90)	0.0004	-1.6
≥80 (n=266)	185.0 (95)	184.8 (75)	183.5 (51)	182.8 (45)	0.3	-0.7
p trend	<0.0001	0.5	0.6	0.8	p interaction	0.003
β‡ for trend	-2.3	-0.3	-0.4	0.3		

*Model adjusted for age (per decade, in each MABP subgroup analysis), mean arterial blood pressure (per 10mmHg, in each age subgroup analysis), gender, body mass index, smoking status and serum glucose.

†CRAE=central retinal arteriolar equivalent, adjusted for venular diameter using the residual method

‡β=regression coefficient

Table 3.1.2. Mean* arteriole adjusted-CRVE† (microns) stratified by age group and mean arterial blood pressure.

Age Group (years)	Mean arterial blood pressure (mmHg) <100 (n=1380)	100-109 (n=1068)	110-119 (n=597)	≥120 (n=301)	p trend	β‡ for trend
<60 (n=981)	224.7 (473)	227.4 (327)	229.0 (120)	228.0 (61)	0.06	0.9
60-69 (n=1237)	225.0 (499)	225.9 (396)	227.5 (237)	225.2 (105)	0.2	0.6
70-79 (n=862)	223.0 (313)	224.5 (270)	224.6 (189)	225.4 (90)	0.2	0.7
≥80 (n=266)	219.4 (95)	218.8 (75)	217.0 (51)	216.7 (45)	0.4	-0.7
p trend	0.4	0.01	0.004	0.01	p interaction 0.05	
β‡ for trend)	-0.4	-1.6	-2.5	-3.1		

*Model adjusted for age (per decade, in each MABP subgroup analysis), mean arterial blood pressure (per 10mmHg, in each age subgroup analysis), gender, body mass index, smoking status and serum glucose

†CRVE=central retinal venular equivalent, adjusted for arteriolar diameter using the residual method

‡β=regression coefficient

Table 3.1.3. Mean* unadjusted CRAE† (microns) stratified by age group and mean arterial blood pressure

Age Group (years)	Mean arterial blood pressure (mmHg) <100 (n=1380)	100-109 (n=1068)	110-119 (n=597)	≥120 (n=301)	p trend	β‡ for trend
<60 (n=981)	203.9 (473)	195.5 (327)	191.7 (120)	185.9 (61)	<0.0001	-5.3
60-69 (n=1237)	198.9 (499)	193.6 (396)	189.8 (237)	185.9 (105)	<0.0001	-3.5
70-79 (n=862)	192.3 (313)	188.8 (270)	185.9 (189)	182.0 (90)	<0.0001	-3.0
≥80 (n=266)	183.8 (95)	180.5 (75)	179.2 (51)	174.7 (45)	0.006	-2.6
p trend	<0.0001	<0.0001	0.0006	0.004	p interaction 0.05	
β‡ for trend	-5.5	-3.8	-3.5	-3.6		

*Model adjusted for age (per decade, in each MABP subgroup analysis), mean arterial blood pressure (per 10mmHg, in each age subgroup analysis), gender, body mass index, smoking status and serum glucose.

†CRAE=central retinal arteriolar equivalent

‡β=regression coefficient

Table 3.1.4. Mean* unadjusted CRVE† (microns) stratified by age group and mean arterial blood pressure

Age Group (years)	Mean arterial blood pressure (mmHg) <100 (n=1380)	100-109 (n=1068)	110-119 (n=597)	≥120 (n=301)	p trend	β‡ for trend
<60 (n=981)	229.8 (473)	227.7 (327)	228.1 (120)	224.0 (61)	0.003	-1.8
60-69 (n=1237)	227.9 (499)	226.3 (396)	226.2 (237)	222.2 (105)	0.04	-1.1
70-79 (n=862)	222.7 (313)	222.7 (270)	220.9 (189)	221.4 (90)	0.4	-0.5
≥80 (n=266)	214.5 (95)	213.4 (75)	209.5 (51)	208.3 (45)	0.06	-1.8
p trend	<0.0001	0.0003	<0.0001	0.0008	p interaction 0.8	
β‡ for trend	-2.9	-2.7	-4.2	-4.4		

*Model adjusted for age (per decade, in each MABP subgroup analysis), mean arterial blood pressure (per 10mmHg, in each age subgroup analysis), gender, body mass index, smoking status and serum glucose.

†CRVE=central retinal venular equivalent

‡β=regression coefficient

Chapter 4: Prevalence and associations of enhanced retinal arteriolar light reflex: a new look at an old sign

Abstract

Purpose: To assess the prevalence, associated risk factors and prognosis (mortality) of the enhanced retinal arteriolar light reflex sign in an older Australian population.

Design and participants: Population-based, cross-sectional study comprising 3654 participants (82.4% response) aged 49+ years from the Blue Mountains region, Australia.

Methods: Retinal photographs of participants were graded for presence and severity of the enhanced arteriolar light reflex sign, by comparison with standard photographs. Associations with systemic factors (subject-specific) and ocular variables (eye-specific) were assessed by logistic regression. Mortality data were obtained using the Australian National Death Index. Hazard ratios were calculated using Cox regression.

Main Outcome Measures: Prevalence of enhanced arteriolar light reflex and associations with demographic variables (age, sex), blood pressure, blood parameters, health risk behaviours, cataract, retinal vessel wall signs, retinopathy and 10-year incident mortality.

Results: The enhanced arteriolar light reflex sign was found in 1053 participants (31.7%, including 28.8% graded as mild and 2.9% as marked). Prevalence decreased with age (36.0%, 37.7%, 28.0% and 18.8% for age groups <60, 60-69, 70-79 and 80+ years, respectively, $p_{(trend)}<0.0001$), odds ratio, OR, 0.78, 95% confidence interval, CI, 0.72-0.85 per decade. Persons with cataract were less likely to have mildly enhanced light reflex (OR 0.74, CI 0.64-0.87). After multivariate adjustment, mildly enhanced light reflex was significantly associated with serum glucose (OR 1.11 per SD increase), cholesterol (OR 1.11), low-density lipoprotein (OR 1.55), triglycerides (OR 1.11), platelets (OR 0.89) and body mass index (OR 1.12). Markedly enhanced light reflex was significantly associated with mean arterial blood pressure (OR 1.24), heavy alcohol consumption (OR 2.66, 40+ grams alcohol per day) and serum glucose (OR 1.16). Strong associations were demonstrated between presence of mildly

enhanced light reflex and either arterio-venous nicking (OR 3.12) or retinopathy (OR 1.96). There was no association between mild or marked enhanced light reflex and either all-cause or vascular mortality.

Conclusions: In this older population, the enhanced retinal arteriolar light reflex sign was a relatively common finding. Although some associations of this sign with vascular risk factors were found, only a marked level of enhanced light reflex was correlated with elevated blood pressure, but not with poor survival.

Introduction

The ophthalmoscopic appearance of a centralized light reflection from the surface of retinal arterioles has been associated with many descriptive terms including "central arteriolar light reflex," "arteriolar light streak," "blood vessel wall reflection," "copper-wiring" and "silver-wiring"[256-258]. The use of the term "arteriolar light reflex" by some authors[259-261] accurately describes this phenomenon, without implying causal mechanisms, and is thus adopted in this article. Alterations to the retinal arteriolar light reflex, specifically changes in its width and increased intensity of the reflex have been incorporated into diverse classification schemes describing hypertensive retinopathy, the fundoscopic appearance of systemic hypertension, since the 1800's[8;257;262;263]. The inclusion of arteriolar light reflex changes was based mainly on observations using direct ophthalmoscopy [262;264-266]. Although the assessment of such alterations to the arteriolar light reflex in direct ophthalmoscopy examinations was considered relatively subjective and unreliable[267-269], many previous reports suggested a link between the enhanced arteriolar light reflex sign and systemic vascular diseases including hypertension and coronary artery disease[260;262;265;270].

Recent developments in the assessment of microcirculation structural changes from retinal photographs have allowed a more precise detection of these microvascular signs in population-based studies[22;233]. Using these photographic grading methods, retinal vessel wall signs including focal arteriolar narrowing, arterio-venous (AV) nicking and changes in retinal vascular calibre were found to be independent markers of systemic cardiovascular, cerebrovascular and ocular vascular events[8;271]. To our knowledge, however, no population-based data have been presented on the prevalence and risk factors of enhanced retinal arteriolar light reflex. The purpose of this study was to report the prevalence of this sign in an older

Australian population, and to assess associated risk factors and prognosis (mortality), using a standardised method of ascertainment.

Methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision, common eye diseases and other health outcomes in an urban predominantly Caucasian population aged 49 years or older. The baseline study, conducted during 1992-4, examined 3654 eligible potential participants living in two postcode areas in the Blue Mountains, west of Sydney, Australia (82.4% response). This study was conducted in accordance with recommendations of the Declaration of Helsinki and was approved by the Western Sydney Area Human Ethics Committee. Written, informed consent was obtained from all participants. Details of recruitment methods were previously described[211;237].

Retinal Grading

At baseline examinations, 30° stereoscopic retinal photographs of both eyes were taken of the macula and optic disc, plus non-stereoscopic photographs of four other retinal fields (temporal, nasal, and both upper and lower vascular arcades), after pupil dilation, using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany). Retinal photographs of both eyes were assessed by graders, masked to participant characteristics, for presence of none, questionable, mild or marked enhancement of the retinal arteriolar light reflex, using a similar grading technique described for other focal retinal vessel abnormalities[233;272]. Briefly, presence of mildly or markedly enhanced light reflex was graded from 35mm slides of both eyes using a light box (Kelvin rating approximately 6200 degrees) and Donaldson stereoscopic viewer with x5

magnification. Photographic standards were selected by a retinal specialist (PM) from the slide set of the Modified Airlie House Classification of Diabetic Retinopathy and BMES participant photographs. Mild enhancement of the retinal arteriolar light reflex was defined as the presence of a reflex with a sharp margin, one-third the width of the arteriolar vessel and consistently present (without interruption) over at least two-thirds the length of the arteriolar sector visible in the field. The width of the reflex was compared with that in the mildly enhanced retinal arteriolar light reflex photographic standard (Figure 3.1.3). Marked enhancement of the retinal arteriolar light reflex was defined as presence of a reflex whose width was greater than one-third the arteriolar wall width, irrespective of its intensity but consistently present over at least two-thirds the length of the arteriolar sector visible in the field. The grading of markedly enhanced arteriolar light reflex was required to be similar to or more obvious than the markedly enhanced retinal light reflex photographic standard (Figure 3.1.4). Any enhancement of the arteriolar light reflex thus included both mild and marked levels. Although methods used for grading other focal vascular abnormalities have excluded the zone within one half disc diameter circumference from the optic disc, grading for the presence of an enhanced light reflex did not exclude this zone due to the requirement to assess consistency of the reflex along the whole course of arterioles. Grades were adjudicated by a senior researcher, and for equivocal or difficult cases, by a retinal specialist (PM). The intra-grader reliability was assessed, with simple kappa statistic of 0.75 and weighted kappa statistic of 0.84.

Focal arteriolar narrowing, AV nicking (nicking) and presence of retinopathy lesions (microaneursyms, haemorrhages) were graded in similar fashion utilizing only arterioles located at least one-half of a disc diameter from the optic disc margin and by comparison with standard photographs selected from the set developed for the Modified Airlie House Classification of

Diabetic Retinopathy (photographic standards 2A, 8A, 10A and 14)[232] and the Wisconsin Age-Related Maculopathy Grading System (cases 54, 8, 58)[233]. Focal arteriolar narrowing was graded as absent/questionable (none/less severe than the standard) or present (equal to or more severe than the standard). AV nicking was graded as present when constriction was evident on both sides of a venule where crossed by an arteriole. AV nicking was graded as absent/questionable, mild, or severe. The quadratic weighted kappa statistic for intra-grader reliability for detection of focal arteriolar narrowing and AV nicking was 0.80 and 0.87, respectively. The grading of non-diabetic retinopathy lesions has previously been reported[234].

Demographic, lifestyle, blood and ocular variables

A standardised interview and examination was performed on all participants during 1992-94. Questions were asked regarding lifestyle factors, including smoking and alcohol consumption. The latter was classified by the number of standard alcohol drinks consumed (defined as 10 grams or 12.5 millilitres of alcohol), according to the Australian National Health & Medical Research Council classification[222]. A medical history (including physician-diagnosed history of stroke and myocardial infarction) was taken. A single measure of systolic and diastolic blood pressure using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Mean arterial blood pressure (MABP) was defined as 0.33 x systolic blood pressure + 0.67 x diastolic blood pressure. Diabetes was defined either from past history of diabetes with current diabetes treatment, or fasting plasma glucose (FPG) ≥7.0mmol/L at examination, according to the most recent World Health Organization diabetes classification scheme[224]. Fasting blood samples were taken and processed for the following blood parameters: haemoglobin, including mean corpuscular volume and mean corpuscular haemoglobin level, white cell count, platelet level, creatinine, glucose, total cholesterol, triglycerides, HDL-

cholesterol and fibrinogen. Blood samples were analysed at the Institute of Clinical Pathology & Medical Research, Westmead Hospital. Body-mass index was calculated as weight (kg)/height $(m)^2$. Low-density lipoprotein (LDL) levels were calculated using the Friedewald equation[273]. Cataract was defined to include participants with nuclear, cortical and posterior subcapsular cataract. Hyperopia was defined as spherical equivalent refraction (SER) greater than one dioptre, and myopia as SER less than one dioptre. The 10-year mortality data from Blue Mountains Eye Study participants were obtained via data linkage with Australian National Death Index (NDI) database in August 2005. The sensitivity and specificity of Australian NDI data has been estimated to be 93.7% and 100 % for all-cause deaths, and 92.5% and 89.6% for cardiovascular deaths [227;228].

Statistical Methods

Statistical analyses were performed using Statistical Analysis System (V8.2, SAS Institute, Cary, NC). Analyses were conducted using person-specific data, so that a participant was classified as having mildly or markedly enhanced retinal arteriolar light reflex, based on the grade in the worse eye. The age-adjusted prevalence of mildly or markedly enhanced arteriolar light reflex is reported, with p-values for trend across age groups calculated using the Mantel-Haenszel chi-square statistic. Logistic regression models were constructed to assess associations between age, gender, history of myocardial infarction or stroke, lifestyle factors, blood parameters, refractive error and presence of any (binomial) or either mildly or markedly enhanced light reflex (multinomial). Analyses for associations with cataract, focal arteriolar *narrowing, AV nicking, and retinopathy in persons without diabetes, were performed using eye-*specific data in generalized estimating equation models. Cox regression methods were used to assess the relationship between the presence of mildly or markedly enhanced light reflex and

mortality, adjusting for age, gender, body mass index, presence of hypertension or use of antihypertensive medication, smoking status and socio-economic status. Odds ratios (OR), hazard ratios (HR), 95% confidence intervals (CI) and adjusted p values for trend are presented.

Results

Of 3654 baseline BMES participants, 3520 (96.3%) had photographs gradable in at least one eye and data available for this study, after excluding 40 persons with missing blood pressure data and 134 persons with missing photographs (overlap in 28 persons). The retinal arteriolar wall reflex was graded as mildly enhanced in 1052 participants (29.9%) and markedly enhanced in 105 participants (3.0%). Combining both eyes, 460 persons (13.1%) had mildly enhanced light reflex bilaterally and only 8 persons (0.2%) had markedly enhanced light reflex bilaterally. Table 3.1.5 shows the prevalence of mildly or markedly enhanced light reflex, stratified by age and gender. The prevalence of mildly enhanced reflex declined with increasing age (p value for trend <0.0001) in both men and women. The overall person-specific prevalence of any (mild or marked) enhancement in arteriolar light reflex by age is shown in Figure 3.1.5.

Mildly enhanced arteriolar light reflex was less frequent if participants had any type of cataract (age-sex adjusted, eye-specific, OR, 0.74, CI 0.64-0.87), but no similar relationship was present in cases with markedly enhanced light reflex (OR 1.10, CI 0.78-1.57). Mildly enhanced light reflex was more frequently observed in myopic (SER <-1.0 dioptres) (right eye, age-sex adjusted OR 1.18, CI 1.02-1.37), but not in hyperopic (SER >1.0 dioptres) eyes (age-sex adjusted OR 1.00, CI 0.93-1.07). Results were similar for left eyes.

Table 3.1.6 shows factors associated with presence of (mildly or markedly) enhanced retinal arteriolar light reflex, after adjusting for age and sex. In the multinomial model for three outcome levels, age, presence of diabetes, serum glucose level, serum cholesterol, triglyceride or LDL level, haemoglobin or platelet level and body mass index were significantly related to presence of a mildly enhanced reflex. Mean arterial blood pressure, systolic blood pressure, untreated hypertension status, serum glucose level, and consumption of 4 or more standard drinks (40+ grams of alcohol) per day was significantly related to presence of a markedly enhanced light reflex. Analyses of any (mildly or markedly) enhanced light reflex showed similar associations to those for mildly enhanced light reflex (data not shown).

Table 3.1.7 shows findings from the multivariate regression model that incorporated statistically significant variables from the age- sex-adjusted models. Presence of cataract remained significantly associated with absence of mildly enhanced light reflex in the multivariable model (OR 0.79, CI 0.66-0.95). In persons without cataract, body mass index (OR 1.12, CI 1.01-1.24), triglyceride level (OR 1.11, CI 1.01-1.22) and platelet level (OR 0.89, CI 0.81-0.99) were also significantly related to mildly enhanced light reflex, in addition to age, serum glucose, cholesterol and LDL levels. Strong associations were found between AV nicking and the presence of any (mild or marked) enhanced arteriolar light reflex, and retinopathy and mildly enhanced light reflex (Table 3.1.8).

There were 1132 deaths (32%) in those with gradable photographs after 10-years follow-up, 279 (7.9%) were deaths from cardiovascular causes and 103 (2.9%) were deaths from cerebrovascular causes. After adjusting for age, gender, body mass index, presence of hypertension or use of antihypertensive medication, smoking status and socio-economic status,

no significant relationship was found between presence of enhanced light reflex and either all-cause mortality (mild level HR 0.98 CI, 0.85-1.13, marked level HR 1.07 CI, 0.74-1.55) or vascular (cardiovascular and cerebrovascular) mortality (mild level HR 1.11 CI, 0.88-1.40, marked level HR 1.29 CI, 0.70-2.36).

Discussion

We used a standardised grading protocol to assess the enhanced retinal arteriolar light reflex sign in our population. This sign was common with an overall prevalence of 32% among persons aged 50 years or older. We found an inverse relationship between age and presence of mildly enhanced light reflex, but there was no age-related association with a markedly enhanced reflex. Persons with cataract were also less likely to have mildly enhanced light reflex. These two findings suggest that both age and cataract may impair the visibility and recognition of a mild level of the enhanced arteriolar light reflex sign. In contrast to previous observations,[256;268] myopic participants were more likely to have mildly enhanced retinal arteriolar light reflex. After multivariable adjustment, serum glucose, cholesterol and LDL levels were positively associated with an increased likelihood of presence of mildly enhanced light reflex. Body mass index, triglycerides and platelets were also statistically significantly associated with this sign in participants without cataract. Both higher blood pressure and alcohol consumption were associated with the presence of a markedly enhanced light reflex. Other retinal vascular signs, AV nicking, and retinopathy in persons without diabetes, were strongly related to presence of any (either mildly or markedly) or mildly enhanced light reflex, respectively.

Recent studies[260;270] have incorporated 'broadening of the light reflex', together with 'copper-wire' and 'silver-wire' changes to arterioles into a classification system for retinal arteriolar sclerosis with a ranking order, adapted from the Schie scheme[257]. Findings from these studies suggested that the higher the ranking in the classification of reflex severity, the greater the severity and extent of coronary vessel atherosclerosis. However, few empirical data exist supporting the concept that light reflex broadening to 'copper-wiring' or 'silver-wiring' represent sequential disease severity stages, nor do any definitive pathological correlates of 'copper-' and 'silver-wiring' exist[263;269]. Use of ophthalmoscopy to detect this sign has been shown to be unreliable, with high levels of inter and intra-observer variability[267;274].

Historically, enhancement of the retinal arteriolar light reflex was thought to indicate reflection from sclerotic arteriolar walls[257;266;275]. Several empirical and theoretical studies[268;276;277] have alternately demonstrated that the reflex probably emanates from the column of densely packed erythrocytes coursing through the retinal microvasculature, viewed through the normally transparent blood vessel wall. Brinchmann-Hansen et al proposed that the light reflex was due to reflected light from the convex blood cell column, with the 'width' of the light reflex primarily a function of the calibre of the column and the relatively slow velocity of blood flow within the microvasculature. The slower velocity of flow, which produces less dense packing of erythrocytes, the rougher and more reflective the blood column surfaces. Since reflection depends upon an abrupt change in refractive index across a surface[278], the 'intensity' of the reflex was thought to be a result of the difference in refractive index between erythrocytes and plasma. Brinchmann-Hansen further suggested that the reflex was unlikely to result from optical aberration, resolution or focusing errors of the fundus camera, and considered that while the width of the reflex was a more reliable measure, its intensity could be reduced by camera

defocus[268]. It remains unclear whether and how refractive index changes to the vessel wall as a result of structural changes from arteriolar sclerosis contribute to alterations of the arteriolar light reflex.

To our knowledge, this is the first study to examine the enhanced retinal arteriolar light reflex in a population-based sample using a reproducible, photographic grading method to assess this sign. Brinchmann-Hansen et al, studying a small sample (n=66), found a greater reflex intensity from older persons than younger persons, but reported no relationship between age and reflex width[279]. However clinical observations[258] have noted striking reflections from the arterioles of younger persons. The age-related decrease in prevalence of mildly enhanced arteriolar light reflex in older persons may be partly explained by the negative association between the mild light reflex and the presence of cataract. Alternately, early age-related changes in the transparency or density characteristics of vessel walls could also reduce differences in refractive indices or increase light scattering, so diminishing visibility of the reflex[279].

We found that elevated blood pressure was related to presence of markedly enhanced arteriolar light reflex, consistent with the previous report of association with systolic blood pressure level by Brinchmann-Hansen[279]. Microvasculature changes resulting from hypertension, either changes to vessel wall transparency (sclerosis), or changes in hemodynamic flow and velocity from microvascular rarefaction,[280;281] or both, may contribute to alterations in visibility of the reflex. The association of enhanced light reflex with AV nicking and retinopathy, rather than focal arteriolar narrowing suggests that the reflex may indicate long-standing hypertension, but may not necessarily reflect current blood pressure levels. Previous literature[8] has suggested that

focal arteriolar narrowing is related to current but not previous blood pressure levels, whereas AV nicking is related to past blood pressure measurements.

Increasing serum glucose, cholesterol, triglyceride, LDL level, body mass index, and alcohol consumption are also well-known vascular risk factors. It is unclear how these risk factors influence the development of enhanced arteriolar light reflex via either vascular dynamics or changes in refractive index, or both.

Strengths of our study include its large population-based sample and its attempt to standardize the grading of alterations in retinal arteriolar light reflex from fundus photographs. We emphasized the width of the light reflex over light intensity, given the possible variation of light intensity associated with camera focus and media transparency. Despite this attempt, any assessment of this light reflex is ultimately subjective, and did not use modern computerised microdensitometry techniques. Apart from mortality, we also only assessed cross-sectional associations. The possibility that we have inadequately adjusted for confounders cannot be ruled out, in particular the use of a single blood pressure measurement may not accurately reflect 'usual' blood pressure and may explain our negative findings with the mild reflex.

In conclusion, we found that the sign of enhanced retinal arteriolar light reflex can be fairly reliably assessed and was more frequently found in younger than older persons. The marked level of this sign was associated with hypertension, and both the mild and marked levels were associated with some traditional vascular risk factors such as elevated blood lipids and other signs indicating retinal microvasculature structural change (AV nicking and retinopathy). However, we demonstrated that presence of the enhanced arteriolar light reflex did not predict

mortality, and that only the marked level of this sign is appropriate as a marker of hypertensive retinopathy. Further studies are needed to confirm its limited prognostic significance.

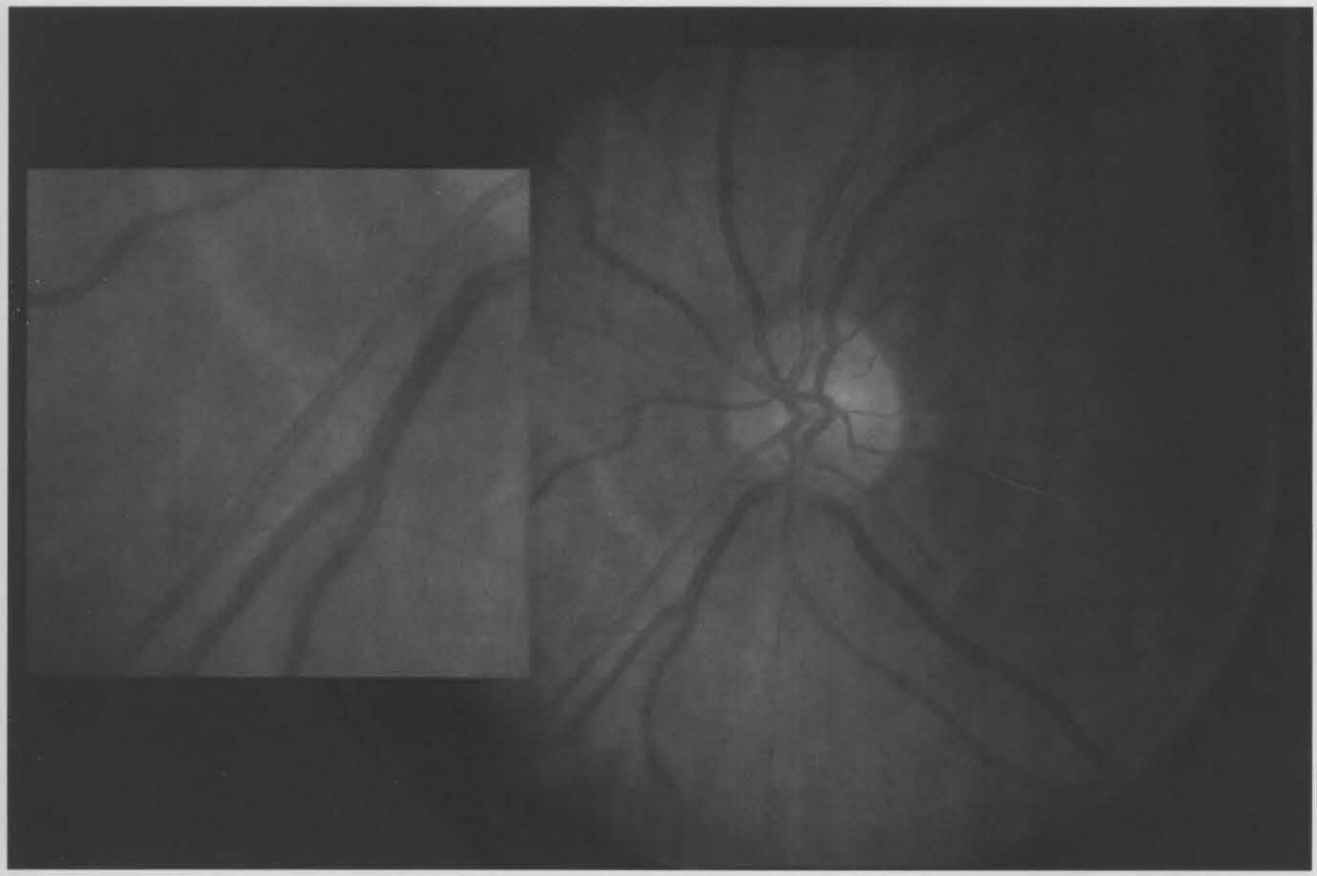

Figure 3.1.3. Standard photograph for mild enhancement of the retinal arteriolar light reflex.

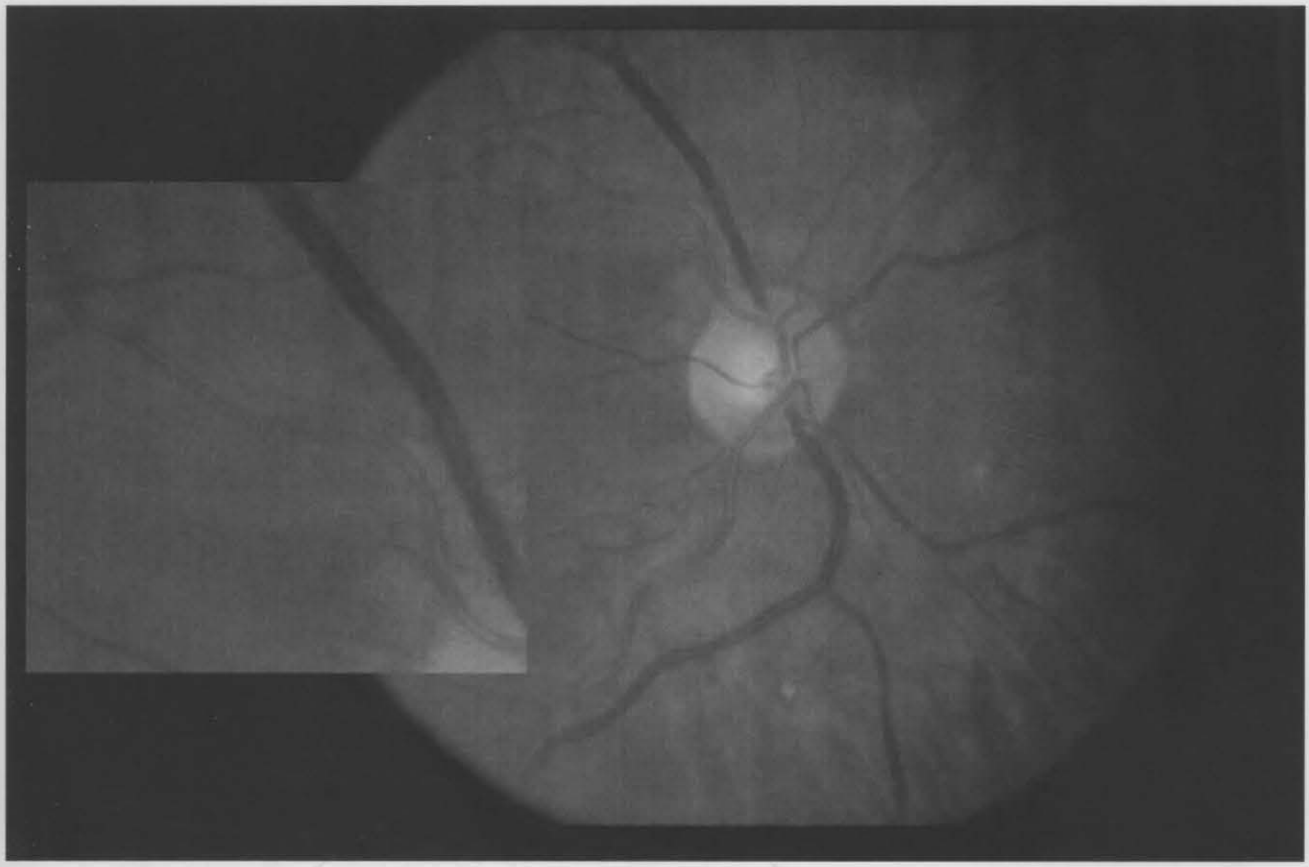

Figure 3.1.4. Standard photograph for marked enhancement of the retinal arteriolar light reflex.

Table 3.1.5. Frequency distribution of mild, marked or any* enhanced arteriolar light reflex, by age and gender in the Blue Mountains Eye Study population

Characteristic	All Ages (%) n=3520	Age Group, years <60 (%) n=996	60-70 (%) n=1264	70-80 (%) n=925	>80 (%) n=335	p for trend†
			men			
	n=1986	n=563	n=690	n=533	n=200	
None	1041 (30)	274 (28)	352 (28)	304 (33)	111 (33)	
Mild	447 (13)	146 (15)	206 (16)	71 (7.7)	24 (7.2)	<0.0001
Marked	46 (1.3)	13 (1.3)	16 (1.3)	17 (1.8)	0 (0)	0.6
Any*	493 (14)	159 (16)	222 (18)	88 (9.5)	24 (7.2)	<0.0001
			women			
	n=1534	n=433	n=574	n=392	n=135	
None	1322 (38)	363 (36)	436 (33)	362 (39)	161 (48)	
Mild	605 (17)	180 (18)	237 (18)	151 (16)	37 (11)	0.0004
Marked	59 (1.7)	20 (2.0)	17 (1.3)	20 (2.2)	2 (0.6)	0.3
Any*	664 (19)	200 (20)	254 (19)	171 (18)	39 (12)	0.0002

*Any = combining mild and marked groups

†p-value for trend comparing percentages for presence of mild, marked or any enhanced arteriolar light reflex

Figure 3.1.5. Prevalence of a mild or marked enhanced arteriolar reflex, by age

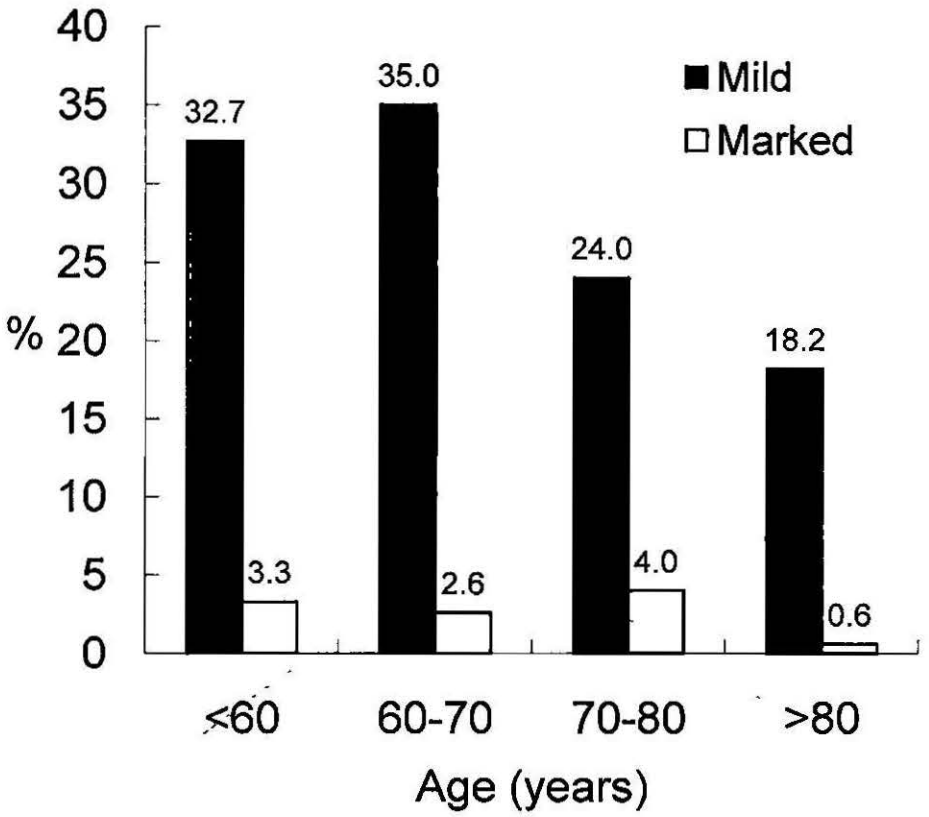

Table 3.1.6. Age- and sex-adjusted associations with presence of a mild or marked enhanced arteriolar light reflex

Factor			Mild	Marked
	No. at Risk	No. Affected (%)	OR (95% CI)*	OR (95% CI)*
Age (per decade)			0.77 (0.72-0.84)	0.83 (0.67-1.02)
Gender (female)			1.07 (0.93-1.24)	1.02 (0.69-1.51)
Hypertensive status†				
Normotensive	1920	622 (32)	1	1
Controlled hypertension	674	221 (33)	1.09 (0.90-1.33)	1.04 (0.60-1.80)
Uncontrolled hypertension	453	161 (36)	1.38 (1.10-1.73)	1.17 (0.60-2.25)
Untreated hypertension	461	149 (32)	1.07 (0.85-1.35)	1.96 (1.16-3.31)
Presence of diabetes	267	103 (39)	1.43 (1.09-1.86)	1.16 (0.55-2.43)
History of myocardial infarction	325	114 (35)	0.87 (0.73-1.03)	0.58 (0.33-1.03)
History of stroke	180	71 (39)	0.88 (0.72-1.07)	0.55 (0.27-1.15)
MABP‡ (per SD)§			1.05 (0.97-1.13)	1.28 (1.06-1.54)
Systolic blood pressure§			1.04 (0.96-1.12)	1.35 (1.12-1.63)
Diastolic blood pressure§			1.04 (0.97-1.12)	1.16 (0.96-1.41)
Serum glucose§			1.14 (1.06-1.23)	1.24 (1.07-1.42)
Serum creatinine§			1.01 (0.92-1.10)	1.03 (0.82-1.29)
Serum cholesterol§			1.10 (1.01-1.19)	1.16 (0.94-1.42)
Serum triglyceride§			1.13 (1.05-1.22)	1.11 (0.92-1.34)
Serum LDL§ ‖			1.45 (1.06-1.99)	1.52 (0.66-3.50)
Serum HDL§ #			0.93 (0.86-1.01)	1.06 (0.86-1.32)

*OR (95% CI) = odds ratio (95% confidence interval)

†Number at risk adds to 3508; 40 missing blood pressure data, 134 missing photos (28 overlap)

‡Mean arterial blood pressure

§Per standard deviation increase

‖LDL = low density lipoprotein

#HDL = high density lipoprotein

**MCV = mean corpuscular volume

††MCH = mean corpuscular haemoglobin

‡‡1 drink per day = 10 grams or 12.5 millilitres of alcohol per day

Table 3.1.6. continued.

Factor			Mild	Marked
	No. at Risk	No. Affected (%)	OR (95% CI)*	OR (95% CI)*
White cell count§			1.04 (0.96-1.12)	1.05 (0.86-1.29)
Haemoglobin§			1.12 (1.02-1.22)	1.13 (0.89-1.44)
MCV§ **			1.03 (0.96-1.12)	1.12 (0.91-1.39)
MCH§ ††			1.06 (0.98-1.14)	1.16 (0.98-1.14)
Platelet count§			0.92 (0.85-1.00)	0.89 (0.72-1.11)
Serum fibrinogen§			0.98 (0.90-1.06)	1.11 (0.97-1.27)
Body mass index§			1.13 (1.05-1.22)	1.03 (0.85-1.26)
Ex-smoker	1225	405 (33)	1.18 (0.94-1.32)	1.13 (0.72-1.76)
Current smoker	527	194 (37)	1.19 (0.96-1.47)	1.07 (0.60-1.92)
Alcohol intake				
<1 drink per day‡‡	1189	374 (31)	0.92 (0.77-1.11)	1.35 (0.81-2.25)
1-4 drinks per day	959	340 (36)	1.17 (0.96-1.42)	1.35 (0.79-2.34)
≥4 drinks per day	124	51 (41)	1.37 (0.91-2.06)	2.72 (1.10-6.70)

*OR (95% CI) = odds ratio (95% confidence interval)

†Number at risk adds to 3508; 40 missing blood pressure data, 134 missing photos (28 overlap)

‡Mean arterial blood pressure

§Per standard deviation increase

‖LDL = low density lipoprotein

#HDL = high density lipoprotein

**MCV = mean corpuscular volume

††MCH = mean corpuscular haemoglobin

‡‡1 drink per day = 10 grams or 12.5 millilitres of alcohol per day

Table 3.1.7. Multivariate-adjusted associations* with presence of a mild or marked enhanced arteriolar light reflex

	Mild	Marked
Factors	OR (95% CI)†	OR (95% CI)†
Age (per decade)	0.76 (0.69-0.84)	0.88 (0.69-1.10)
Presence of diabetes#	1.22 (0.91-1.63)	
MABP‡ (per SD)§**		1.24 (1.02-1.51)
Hypertensive status**		
Normotensive		1
Controlled hypertension		0.91 (0.50-1.65)
Uncontrolled hypertension		0.90 (0.45-1.84)
Untreated hypertension		1.91 (1.11-3.27)
Systolic blood pressure§ **		1.30 (1.07-1.59)
Serum glucose§ #	1.11 (1.02-1.21)	1.16 (1.01-1.33)
Serum cholesterol§ ††	1.11 (1.02-1.21)	
Serum triglyceride§††	1.06 (0.97-1.15)	
Serum LDL§ ‖ ††	1.55 (1.12-2.15)	
Haemoglobin§	1.04 (0.96-1.13)	
Platelet count§	0.94 (0.86-1.02)	
Body mass index§	1.07 (0.98-1.16)	
Alcohol intake		
<1 drink per day‡‡		1.45 (0.86-2.47)
1-4 drinks per day		1.28 (0.73-2.27)
≥4 drinks per day		2.66 (1.11-6.37)

*Adjusted for age, sex, cataract, refractive error and all variables significant in the age- sex-adjusted models; includes subjects with cataract

†OR (95% CI) = odds ratio (95% confidence interval)

‡Mean arterial blood pressure

§Per standard deviation increase

‖LDL = low density lipoprotein

#Categorical diabetes status and serum glucose alternately placed in model

**Categorical hypertensive status, MABP and systolic blood pressure alternately placed in model

††Serum cholesterol with triglyceride, and serum LDL alternately placed in model

‡‡1 drink per day = 10 grams or 12.5 millilitres of alcohol per day

Table 3.1.8. Retinal vessel wall signs, retinopathy and associations* with presence of a mild or marked enhanced arteriolar light reflex

Retinal Signs	Mild		Marked	
	n (%)	OR (95% CI)†	n (%)	OR (95% CI)†
Presence of focal arteriolar narrowing	73 (27)	0.79 (0.56-1.11)	4 (1.5)	0.54 (0.16-1.86)
Presence of arterio-venous nicking	733 (43)	3.12 (2.73-3.57)	70 (4.1)	1.77 (1.19-2.63)
Presence of retinopathy‡	140 (40)	1.96 (1.54-2.50)	14 (4.0)	1.40 (0.72-2.73)

*Eye-specific model; adjusted for age, gender, mean arterial blood pressure, body mass index, smoking, serum glucose and cholesterol

†OR (95% CI) = odds ratio (95% confidence interval)

‡Microaneursyms and haemorrhages only, excludes persons with diabetes

Part 3.2

Diet and the Retinal Microvasculature

Chapter 5: Frequency of fish consumption, retinal microvascular signs and vascular mortality

Abstract

Objectives: Fish consumption has established cardiovascular and cerebrovascular benefits, but its effects on microvascular structure have not been examined in population-based studies. We investigated this association, in relation to vascular mortality in an Australian cohort (1992-2004).

Methods: Of 3654 participants aged 49+ years, 2683 (73%) with available data were included. Retinal arteriolar and venular diameters were measured, and signs of arterio-venous nicking and retinopathy were assessed from digital retinal images. Fish consumption was evaluated using a food frequency questionnaire.

Results: Both wider mean arteriolar diameter ($p=0.002$) and narrower venular diameter ($p=0.02$) were associated with increasing frequency of consuming any or oily fish, after adjusting for cardiovascular risk factors, diet, inflammatory factors and socioeconomic status. This association was mainly present in persons with hypertension. Greater frequency of fish consumption was associated with a reduced prevalence of arterio-venous nicking and a borderline significant trend for reduced retinopathy prevalence. 10-year stroke-related mortality was significantly lower in persons consuming fish at least once per week compared to less frequent consumption (hazard ratio 0.57, 95% CI: 0.35, 0.93).

Conclusions: Recent evidence shows that narrower arterioles and wider venules may predict vascular events. Our new findings suggest that the vascular protective effects of consuming fish could act, in part, by preventing pathological microvasculature change.

Introduction

Regular consumption of even small amounts of fish appears to reduce the risk of incident coronary heart disease mortality and stroke.[282;283] Long chain omega-3 polyunsaturated fatty acids are thought to be the principal nutrients responsible. The exact mechanisms and compounds responsible for the protection, however, remain unclear. In coronary heart disease, where inverse associations between fish consumption and related mortality have been observed, there are few data on the benefits of fish consumption against the more relevant outcome, coronary vessel arteriosclerosis.[284] This is relevant, given recent population-based data implicating small vessel dysfunction in both coronary and cerebrovascular disease,[13;285] and debate generated by a recent review of omega-3 fats.[286] Similar data linking fish consumption to microvascular signs could shed light on the potential for cardio- and cerebro-protection from fish in the diet.

Accumulating experimental evidence suggests that microvascular dysfunction precedes the development of cardiovascular diseases such as hypertension.[3] Retinal photographs permit non-invasive in-vivo examination of the microvasculature. Qualitative and quantitative evaluation of signs traditionally attributed to hypertension is now feasible using reproducible computer-assisted methods to assess digitized retinal photographs.[8] Population-based studies have shown that abnormal retinal vessel diameter may predict systemic and ocular vascular and events.[8;287] Both narrower retinal arterioles and wider retinal venules are independent predictors of incident hypertension, stroke and coronary heart disease death.[4;10-13]

To date, population studies have not examined whether consuming fish regularly has beneficial influences on structural retinal microvascular signs shown to predict vascular risk, namely

narrowing of retinal arterioles, widening of retinal venules and focal retinal vessel changes (arterio-venous nicking and retinopathy). We aimed to investigate this association in an older Australian cohort, and to assess long-term influences of consuming fish on vascular mortality.

Materials and Methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision, common eye diseases and other health outcomes in an urban, predominantly Caucasian population aged 49 years or older. The baseline study in 1992-4, examined 3654 eligible residents of two postcodes of the Blue Mountains region, west of Sydney, Australia (82.4% response). Subsequent 5- and 10-year examinations of this cohort have been conducted and long-term mortality assessed.[211;237] The study adhered to recommendations of the Helsinki Declaration and was approved by the Sydney West Area Human Ethics Committee. Written, informed consent was obtained from all participants.

Retinal Photography

Detailed methods for grading the diameters of retinal arterioles and venules are described elsewhere.[22] In brief, at the baseline examination, 30° photographs of macular, optic disc and other retinal fields of both eyes were taken, after pupil dilation, using a Zeiss FF3 fundus camera (Zeiss, Oberkochen, Germany). We used methods developed and found reproducible, by the University of Wisconsin-Madison,[233] to measure the internal diameter of retinal arterioles and venules from the digitised photographs. These were then summarised using established formulas,[28] that account for branching patterns and combine individual vessel diameters into

summary indices, reflecting the mean arteriolar and venular diameters, respectively, for each eye.

Retinal photographs were assessed by masked graders, for presence of arterio-venous nicking, by comparison with standard photographs, as described elsewhere.[22] This sign was considered present when constriction was evident on both sides of a venule where crossed by an arteriole. Its severity was graded as absent/questionable, mild, or severe. Retinopathy lesions in subjects without diabetes were also graded using the modified ETDRS classification of diabetic retinopathy,[235;236] as previously reported.[237] Retinopathy was recorded if any of the following lesions were present: (1) microaneurysms, (2) blot or flame shaped hemorrhages at least 0.5 disc diameter from the disc margin, (3) hard exudates or (4) cotton wool spots.

Dietary Data

A standardised interview and examination was performed and participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular from a Willett questionnaire,[213] that incorporated a nine-category frequency scale and standard portion size estimates. The FFQ had reasonable concurrent validity[214] when validated against 4-day weighed food records collected on three occasions in one year (n=79). For fats, the FFQ was found to show moderate to good agreement for ranking individuals according to their fat intakes, yielding correlation coefficients between 0.4-0.7, and correctly classifying over 70 percent of people within one quintile for all types of fats.[214] Dietary intakes were estimated using Australian Tables of Food Composition (NUTTAB90),[216] and a fatty acid supplement database.[218;219] FFQs giving implausible total energy intakes or with excessive missing items were excluded. We extracted separate data on the frequency of consuming any fish (any species

plus method of preparation) and oily fish (specifically salmon, tuna and sardines). Consumption of long chain omega-3 fatty acids was estimated by summating dietary intakes of eicosapentaenoic acid (EPA 20:5:n-3), docosapentaenoic acid (DPA 22:5n-3) and docosahexaenoic acid (DHA 22:6:n-3).

Demographic, lifestyle and dietary variables

The interview included questions about past medical history, including physician-diagnosed history of stroke and myocardial infarction, and lifestyle factors such as smoking. A single measure of systolic blood pressure (SBP) and diastolic blood pressure (DBP) using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Mean arterial blood pressure (MABP) was defined as 0.33×SBP +0.67×DBP. Severe hypertension was defined using World Health Organisation criteria for hypertension stage II (SBP ≥160 mm Hg and/or DBP ≥100 mm Hg) or in subjects with previously diagnosed hypertension or those using anti-hypertensive medications. Body-mass index was calculated as weight (kg)/height $(m)^2$. Fasting blood samples were processed the same day for: haemoglobin, white cell and platelet counts, glucose, total cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol and fibrinogen by the Institute of Clinical Pathology, Westmead Hospital. Ten-year mortality data was obtained via data linkage with Australian National Death Index (NDI) database in August 2005. The sensitivity and specificity of Australian NDI data has been estimated to be 93.7% and 100 % for all-cause deaths, and 92.5% and 89.6%, respectively, for cardiovascular deaths.[227;228] White cell and platelet count, fibrinogen and smoking status were defined as inflammatory factors.

Statistical Methods

Statistical analyses were performed using Statistical Analysis System (SAS Institute, Cary, NC). Subject intakes were divided into quintiles for each fat nutrient and by frequency of fish consumption (less than once per week, once per week and at least twice per week). Dietary fat variables were adjusted for total energy intake using the Willett residual method.[241] This was also used to assess the effect of nutritional variables on retinal arteriolar or venular diameter, independent of the fellow vessel component. Venule-adjusted arteriolar diameter was defined using linear regression with venular diameter as the independent variable and arteriolar diameter the dependent variable, by calculating residuals, and adding these to the expected mean venular diameter. Arteriole-adjusted venular diameter was similarly defined. The resulting adjusted variables represent the non-shared variance of each vessel diameter, respectively.[11;12] Mean venule-adjusted arteriolar diameter and arteriole-adjusted venular diameter for each fish frequency category or fatty acid quintile was assessed using ANCOVA. Similarly, odds ratios (OR) and 95% confidence intervals (CI) for presence of arterio-venous nicking and retinopathy were calculated using logistic regression. Three models were constructed: Model 1 adjusted for age, gender, MABP, body-mass index, smoking, glucose, cholesterol, white cell and platelet counts, qualifications, self-rated health, past history of heart disease and total vegetable and fat intakes. Model 2 was adjusted for variables in Model 1 plus nutrient variables, vitamins C and E, and beta-carotene. Model 3 further adjusted for haemoglobin and fibrinogen when the dependent variable was mean venular diameter, AV nicking or retinopathy.[247;288] Cox regression was used to assess hazard ratios (HR) with CI from consuming fish or long chain omega-3 fatty acid intakes on either stroke-related or coronary heart disease mortality, after adjusting for age, gender, body mass index, MABP, smoking status, socio-economic status and total energy intake.

Results

Of the 3654 baseline BMES participants, 3267 completed the FFQ and 2897 were sufficiently complete and plausible for analysis. A further 185 participants were excluded because they had missing retinal photographs or poor photographic images precluding vessel measurement, or had retinal diseases confounding measurement of retinal vessel diameter, plus 29 participants who did not answer the fish questions or had missing blood pressure data. Participants without usable FFQ data were more likely to be older (mean age 69.3 years vs. 65.3 years), or current smokers (17.7% vs. 14.2%) than those with usable FFQ's (data not shown). Among those completing the FFQ, AV nicking was present in 48.3% and retinopathy was found in 9.2% of nondiabetic participants. The mean venule-adjusted arteriolar diameter in this population was 187.3 microns and the mean arteriole-adjusted venular diameter was 225.0 microns. Serum cholesterol, white cell and platelet counts, smoking status, qualification level, self-rated health, and the intakes of vegetables and several nutrients, differed between fish consumption strata (Table 3.2.1).

Table 3.2.2 shows mean arteriolar and venular diameters by the frequency of consuming any fish and oily fish, after adjusting for multiple confounders. Increasing frequency of fish consumption was associated with wider mean arteriolar diameter (p for trend 0.002 to 0.003) and narrower mean venular diameter (p for trend 0.02 to 0.003). Table 3.2.3 shows that after stratifying by presence and severity of hypertension, these relationships were mainly evident and statistically significant among hypertensive participants. The difference in range of diameters for both arterioles and venules was greater for oily fish than with overall fish consumption. Stratification indicated that the association between increasing frequency of fish consumption and vessel diameter was mainly driven by, and statistically significant only for persons using anti-hypertensive medication (data not shown). These associations persisted after

replacing the intakes of vegetables with micronutrients in Model 2, or after further controlling for haemoglobin and fibrinogen in Model 3. There was no significant interaction between fish consumption and use of anti-hypertensive medications. Increased fish in the diet was associated with a lower arterio-venous nicking prevalence, for fish consumption at least twice per week vs. less than once per week, OR, 0.82 95% CI, 0.68 to 0.99, p for trend=0.03. It was also associated with a borderline significant trend for lower prevalence of retinopathy in nondiabetic participants, for fish consumption at least twice per week vs. less than once per week, OR, 0.72 95% CI, 0.52 to 0.99, p for trend=0.05, as shown in Table 3.2.4. These trends were similar when consumption of oily fish was considered separately, but were not statistically significant. The associations persisted after accounting for confounders in Models 2 and 3.

Increasing quintiles of dietary long-chain omega-3 fatty acid intakes were associated with statistically significant trends for wider mean arteriolar diameter (5^{th} vs. 1^{st} quintile, p for trend=0.01), narrower mean venular diameter (5^{th} vs. 1^{st} quintile, p for trend=0.02), and decreased prevalence of AV nicking (5th vs. 1st quintile, p for trend=0.02), but not for retinopathy lesions in nondiabetic participants (5th vs. 1st quintile, p=0.9). There was no significant association between greater consumption of saturated, monounsaturated or polyunsaturated fats (by quintile) and retinal vessel diameters or vessel wall signs (data not shown).

This cohort demonstrated a reduction in stroke-related mortality over the subsequent 10 years, associated with the reported consumption of fish at least once per week, and with consumption of increasing quintiles of dietary long-chain omega-3 fatty acids (Table 3.2.5). For fish, the HR was 0.57, CI 0.35 to 0.93, and for fatty acids, the HR was 0.20, CI 0.04 to 0.97. There was,

however, no statistically significant reduction in coronary heart disease mortality; for fish, the HR was 0.89, 95% CI, 0.67 to 1.13, and for fatty acids, the HR was 0.45, 95% CI, 0.18 to 1.17. The stroke mortality associations persisted after also adjusting for retinal vessel diameter and for presence of AV nicking or retinopathy. Among persons younger than 75 years, a 20% change in stroke mortality risk was observed (HR was reduced from 0.57 to 0.75), after including retinal vessel variables in the model.

Discussion

To our knowledge, the frequency of consuming fish has not been linked to microvascular retinal signs in other populations. We found that regular fish in the diet, particularly oily fish, eaten at least twice per week, was associated with slight widening of mean retinal arteriolar diameter and slight narrowing of mean retinal venular diameter. Both are beneficial structural changes found associated with lower risk of cardiovascular and cerebrovascular diseases.[4;10-13] Stratified analyses suggested that this benefit was mainly evident among persons with severe hypertension, particularly those using anti-hypertensive medications. Regular fish in the diet was also associated with reduced prevalence of two important focal microvascular signs, arterio-venous nicking, and retinopathy lesions in nondiabetic persons. The potential for microvascular benefits from fish was supported by similar findings for diets rich in long-chain omega-3 fatty acids, to which fish is a major contributor. These beneficial effects were independent of many confounders, including cardiovascular risk factors, inflammatory variables, socioeconomic status and other dietary constituents.

Many epidemiological studies, randomised clinical trials and meta-analyses, have shown protective effects from regular fish consumption against coronary heart disease

mortality,[283;289;290] stroke[282] and hypertension.[291] A recent clinical review[290] concluded that the benefits of modest fish consumption (1-2 servings/week) outweigh any risks among adults. Our study demonstrates a similar 43% reduction in stroke mortality to the 36% reduction quoted in this meta-analysis[290] and provides a possible mechanism to partly explain the benefit from fish consumption on vascular events. Population studies have shown that narrower arterioles and wider venules predict incident hypertension, coronary heart disease mortality and stroke.[4;10-13]

These new findings suggest that the cardio-protective effects of dietary omega-3 fatty acids may thus be partly mediated by their beneficial effects on the microvasculature. Our finding of a reduction in stroke mortality associated with fish in the diet, and the attenuation of this reduced risk after adjusting for retinal arteriolar or venular diameter among participants aged younger than 75 years, lends support to this speculation.

In stratified analyses, we observed that the beneficial changes in retinal vessel diameter were mainly evident among persons with severe hypertension. This finding is in keeping with that by Geleijnse et al showing that fish oil produced a greater incremental decline in blood pressure in hypertensive than non hypertensive subjects and their speculation that this could result from improved microvascular function.[291] We also documented additive effects on vessel diameter from fish consumption and anti-hypertensive medication, consistent with reports on the combined cardiovascular benefits of diet and anti-hypertensive medication.[292]

It should be recognised that the retinal vessel diameter differences observed between the lowest and highest categories of fish consumption are modest (around only 1% of vessel diameters), and could have arisen by chance alone. It is known, however, that even small reductions in

retinal arteriolar diameter may be associated with moderate changes in blood pressure: for example, each 10mmHg increase in systolic blood pressure has been associated with a 1.1 micron reduction in arteriolar diameter.[51] Since a reduction in blood pressure of even 5mmHg has been considered sufficient to prevent one-third of strokes and one-fifth of coronary events,[293] our findings of a minimum 2.2 micron widening in arteriolar diameter associated with increasing frequency of fish consumption suggest a potentially important role for fish in preventing both hypertension and cardiovascular disease.

There may be several mechanisms operating at a microvascular level by which fish protects against vascular disease. There is now good evidence that the signs of wider retinal venular diameter and arterio-venous nicking have chronic inflammatory contributions.[247;288] Given the importance of inflammation at all stages of coronary artery and systemic atherogenesis[294] it is possible that regular fish consumption attenuates inflammatory processes by antagonising pro-inflammatory cytokines that increase risk of cardiovascular disease.[167] Microvessel disruption and leakiness has been implicated in some forms of coronary plaque rupture as well as in retinopathy.[295] Finally, reduction of blood pressure, improvements in vascular reactivity or endothelial function could be mechanisms operating in observational studies of fish and vascular disease.[296]

We used venule-adjusted arteriolar diameter and arteriole-adjusted venular diameter to control for the shared variance between the two vessel types resulting from the influences of body size and genetic factors. This permitted examination of associations using a relatively independent measure of their diameters and allowed us to avoid potential confounding effects from concordant fellow vessel influences.[11;12]

Strengths of this study include its conduct within a well-defined urban population using a questionnaire that permitted careful assessment of potential confounding variables. The retinal vessel grading was masked, with good intra-grader reliability,[22;28] so that measurement error in the assessment of dietary variables is likely to be non-differential. Indication bias is also unlikely to have influenced our study findings. Although participants diagnosed with hypertension could have reduced their saturated fat intake after diagnosis, they would have been less likely to have increased their fish consumption, given the paucity of knowledge about its benefits available to the general public in the period 1992-4. An important limitation of our study is the cross-sectional analysis of retinal microvascular signs, so that causality cannot be assumed. Another limitation of our study is the use of a single blood pressure measurement, which may not accurately reflect a participant's 'usual' blood pressure. Despite our best efforts to control for socioeconomic factors, incomplete control of important confounding from unmeasured lifestyle and social factors may still exist.

In conclusion, we observed that greater frequency of fish consumption was associated with wider retinal arteriolar diameters, narrower retinal venular diameters and lower prevalence of arterio-venous nicking and nondiabetic retinopathy. These beneficial changes in vessel diameter were mainly observed in persons with hypertension. The long-term protective effects on stroke mortality associated with eating fish were attenuated after incorporating the adjustment for retinal vessel diameters. This finding supports the concept that the vascular-protective effects of diets with increasing serves of fish could, in part, be mediated through beneficial influences on the microvasculature. Prospective studies and intervention trials are needed to confirm these findings.

Table 3.2.1. Characteristics of participants at the baseline Blue Mountains Eye Study examination by frequency of fish* consumption.

Characteristics	Fish Consumption (<1/week) n=1090	Fish Consumption (1/week) n=716	Fish Consumption (≥2/week) n=877	P-value
Continuous Factors	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
Mean age (yrs)	65.6 (9.5)	64.9 (9.1)	65.5 (9.2)	0.22
Mean arterial blood pressure (mmHg)	104 (12.1)	104 (12.3)	104 (12.3)	0.68
Mean body-mass index	26.1 (4.6)	26.1 (4.4)	26.3 (4.6)	0.79
Mean fasting serum glucose (mmol/L)	5.24 (1.5)	5.27 (1.6)	5.22 (1.5)	0.75
Mean fasting serum cholesterol (mmol/L)	6.00 (1.1)	5.96 (1.1)	6.12§ (1.1)	0.007
Mean white cell count ($x10^9$)	6.63 (1.8)	6.40 (1.7)	6.40§ (1.7)	0.002
Mean platelet count ($x10^9$)	266 (64.1)	260 (64.7)	259§ (58.8)	0.01
Mean vegetable intake (grams/day)	402 (198)	435 (173)	489§ (196)	<0.001
Mean total fat intake (grams/day)	76.9 (13.6)	76.6 (13.6)	74.7§ (14.5)	0.001
Mean vitamin E intake (mg/day)	32.4 (103)	43.6 (114)	45.2§ (121)	0.02
Mean vitamin C intake (mg/day)	295 (355)	347 (371)	386§ (417)	<0.001
Mean beta-carotene intake (micrograms/day)	6986 (4445)	7207 (4019)	7961§ (4824)	<0.001
Mean zinc intake (mg/day)	11.7 (2.4)	11.8 (2.0)	11.8 (2.2)	0.42

*Any fish consumption

†Self-rated health described as "fair" or "poor" compared with "excellent" or "good"

‡Any post school qualification (inc. trade certificate, diploma, degree)

§p for trend across fish consumption categories <0.05

||Mantel-Haenszel chi-square test for trend across fish consumption categories, p<0.05

Table 3.2.1. continued.

Characteristics Categorical Factors	Fish Consumption (<1/week) n=1090 % (SD)	Fish Consumption (1/week) n=716 % (SD)	Fish Consumption (≥2/week) n=877 % (SD)	P-value
Male gender (%)	55.8 (0.5)	56.2 (0.5)	56.6 (0.5)	0.80
Current smoker (%)	17.5 (0.5)	15.2 (0.5)	9.9‖ (0.5)	<0.001
Past smoker (%)	33.8 (0.4)	36.8 (0.4)	39.0‖ (0.3)	0.02
Diabetes (%)	7.17 (0.3)	7.40 (0.3)	7.18 (0.3)	0.94
Past history of myocardial infarction (%)	14.9 (0.4)	12.6 (0.3)	16.1 (0.4)	0.54
Past history of stroke (%)	4.68 (0.4)	5.45 (0.4)	3.31 (0.4)	0.33
Self-rated health (%)†	25.8 (0.4)	19.0 (0.4)	21.6‖ (0.4)	0.02
Qualification level (%)‡	47.4 (0.5)	48.0 (0.5)	55.7‖ (0.5)	<0.001

*Any fish consumption

†Self-rated health described as "fair" or "poor" compared with "excellent" or "good"

‡Any post school qualification (inc. trade certificate, diploma, degree)

§p for trend across fish consumption categories <0.05

‖Mantel-Haenszel chi-square test for trend across fish consumption categories, p<0.05

Table 3.2.2. Multivariate adjusted* mean (95% confidence interval) retinal arteriolar and venular diameters for increasing frequency of fish consumption.

Fish	**n**	**Mean arteriolar diameter† (microns)**	**Mean venular diameter‡ (microns)**
Any Fish Consumption			
Less than once per week	1090	186.3 (185.3-187.2)	225.8 (224.8-226.9)
Once per week	716	186.9 (185.4-188.1)	225.6 (224.3-226.8)
At least twice per week	877	188.5 (187.5-189.5)	223.9 (222.9-225.1)
P for trend		0.002	0.02
Oily Fish Consumption§			
Less than once per week	1724	186.6 (185.9-187.3)	225.9 (225.1-226.7)
Once per week	644	187.8 (186.6-189.1)	224.3 (223.1-225.6)
At least twice per week	315	189.2 (187.5-190.9)	223.0 (221.1-224.9)
P for trend		0.003	0.003

*Adjusted for continuous age, gender, mean arterial blood pressure, body-mass index, smoking status, glucose, cholesterol, white cell count, platelet count, qualification level, self-rated health, past history of myocardial infarction and stroke, total vegetable and fat intake

†Arteriolar diameter is adjusted for venular diameter using the residual method

‡Venular diameter is adjusted for arteriolar diameter using the residual method

§Includes salmon, tuna, sardines

Table 3.2.3. Multivariate adjusted* mean (95% confidence interval) retinal arteriolar and venular diameters for increasing frequency of fish consumption, stratified by hypertension†.

Fish	Mean arteriolar diameter‡ (microns)				Mean venular diameter§ (microns)			
	n	Normotension (n=1484)	n	Hypertension (n=1199)	N	Normotension (n=1484)	n	Hypertension (n=1199)
Any Fish Consumption								
Less than once per week	606	188.4 (187.2-189.6)	484	183.8 (182.4-185.2)	606	225.5 (224.5-226.6)	484	226.5 (224.9-228.1)
Once per week	401	188.3 (186.9-189.7)	315	185.2 (183.5-186.9)	401	224.6 (222.9-226.3)	315	225.6 (223.6-227.5)
At least twice per week	477	189.8 (188.5-191.2)	400	186.7 (185.1-188.2)	477	224.7 (222.2-227.2)	400	223.0 (221.2-224.8)
P for trend		0.14		0.008		0.83		0.006
Oily Fish Consumption‖								
Less than once per week	956	188.8 (187.9-189.8)	768	183.8 (182.8-185.0)	956	225.7 (224.6-226.7)	768	226.2 (224.9-227.5)
Once per week	349	188.0 (186.5-189.6)	295	187.2 (185.5-189.0)	349	224.3 (222.7-226.0)	295	224.1 (222.1-226.2)
At least twice per week	179	190.7 (188.5-192.9)	136	187.5 (184.9-190.2)	179	224.3 (221.8-226.7)	136	220.8 (217.8-223.9)
P for trend		0.37		<0.001		0.37		<0.001

*Adjusted for continuous age, gender, mean arterial blood pressure, body-mass index, smoking status, glucose, cholesterol, white cell count, platelet count, qualification level, self-rated health, past history of myocardial infarction and stroke, total vegetable and fat intake

†Hypertension defined by systolic blood pressure>160 and diastolic blood pressure>100

‡Arteriolar diameter is adjusted for venular diameter using the residual method

§Venular diameter is adjusted for arteriolar diameter using the residual method

‖Includes salmon, tuna, sardines

Table 3.2.4. Multivariate adjusted* odds ratio for increasing frequency of fish consumption and presence of retinal vessel wall signs.

Fish	n	% with AV nicking	AV Nicking† OR (95% confidence interval)	% with retinopathy	Retinopathy‡ OR (95% confidence interval)
Any Fish Consumption					
Less than once per week	1090	51.3	**1.00**	10.3	**1.00**
Once per week	716	46.6	0.84 (0.69-1.02)	8.5	0.90 (0.65-1.26)
At least twice per week	877	47.4	0.82 (0.68-0.99)	7.9	0.72 (0.52-0.99)
P for trend			0.03		0.05
Oily Fish Consumption§					
Less than once per week	1724	49.7	1.00	9.3	1.00
Once per week	644	47.7	0.94 (0.77-1.14)	8.9	0.96 (0.70-1.33)
At least twice per week	315	46.0	0.81 (0.63-1.04)	7.9	0.75 (0.47-1.18)
P for trend			0.08		0.23

*Adjusted for continuous age, gender, mean arterial blood pressure, body-mass index, smoking status, glucose, cholesterol, white cell count, platelet count, qualification level, self-rated health, past history of myocardial infarction and stroke, total vegetable and fat intake

†AV nicking=arterio-venous nicking

‡Retinopathy in persons without diabetes

§Includes salmon, tuna, sardines

Table 3.2.5. Hazard ratios (95% confidence intervals) for consumption of fish, intake of long chain omega-3 fatty acids and 10-year risk of vascular death

Fish	**Frequency of consumption**	**n (deaths)**	**Coronary heart disease related death HR (95% CI)**	**n (deaths)**	Stroke related death HR (95% CI)
Any fish consumption	Less than once per week	81	1.00	38	1.00
	Once per week	45	0.88 (0.60-1.28)	11	0.51 (0.26-0.99)
	At least twice per week	58	0.91 (0.64-1.28)	20	0.62 (0.35-1.10)
	At least once per week	103	0.89 (0.67-1.13)	31	0.57 (0.35-0.93)
Long chain omega-3 fats†	Quintile 1	46	1.00	14	1.00
	Quintile 2	42	1.07 (0.67-1.71)	19	1.09 (0.51-2.30)
	Quintile 3	30	0.78 (0.46-1.32)	16	1.21 (0.56-2.61)
	Quintile 4	30	0.80 (0.47-1.37)	9	0.58 (0.23-1.48)
	Quintile 5	36	0.79 (0.48-1.29)	11	0.48 (0.19-1.22)
	Trend (increasing quintiles)		0.45 (0.18-1.17)		0.20 (0.04-0.97)

*Adjusted for continuous age, gender, mean arterial blood pressure, body-mass index, smoking status, qualification level, self-rated health and past history of myocardial infarction and stroke.

†Energy-adjusted using the Willett residual method

Chapter 6: Glycemic index, retinal microvascular signs and vascular mortality

Abstract

Background and purpose: It is unclear whether diets with high glycemic index (GI) and low cereal fibre (CF) are associated with greater risk of stroke. We aimed to assess the relationship between dietary GI and CF content, retinal microvasculature changes and stroke-related mortality.

Methods: Population-based cohort, 49+ years, examined at baseline (1992-4). At baseline, participants completed validated food frequency questionnaires. Mean GI was calculated using an Australian database. Retinal arteriolar and venular diameters were measured from photographs. Mortality data were derived using the Australian National Death Index.

Results: Over 13 years, 95 of 2897 participants (3.5%) died from stroke. Increasing GI (hazard ratio, HR, 1.91, 95% CI, 1.01-3.47, highest vs. lowest tertile) and decreasing CF (HR, 2.13, 95% CI, 1.19-3.80, lowest vs. highest tertile) predicted greater risk of stroke death, adjusting for multiple stroke risk factors. Persons consuming food in the highest GI tertile and lowest CF tertile had a 5-fold increased risk of stroke death (HR 5.06, 95% CI, 1.67-15.22). Increasing GI and decreasing CF were also associated with retinal venular calibre widening ($p_{trend}<0.01$). Adjustment for retinal venular calibre attenuated stroke death risk associated with high GI by 50%, but did not affect the risk associated with low CF consumption.

Conclusions: High GI and low CF diets predict greater stroke mortality and wider retinal venular calibre. The association between high GI diet and stroke death was partly explained by GI effects on retinal venular calibre, suggesting that high GI diet may produce deleterious anatomical changes in the microvasculature.

Introduction

There is increasing evidence that postprandial or post-challenge glycaemia is an important, independent risk factor for stroke.[297] Dietary glycemic index (GI) is a commonly used marker of the postprandial blood glucose response. This index ranks carbohydrates, according to their effect on blood glucose [298].

Only a few studies have examined the relationship of GI and risk of stroke,[299-301] and pathophysiological mechanisms underlying this potential association are unclear. It has been postulated that high GI diets might lead to small vessel dysfunction - an important precursor of stroke and other cardiovascular diseases.[3;13;285;299;302]. The formation of advanced glycation end products, glycaemia-induced oxidative stress or the effects of inflammatory intermediaries have all been proposed as possible factors mediating the effects of higher GI on small vessels.[302-306]

The retinal microvasculature shares similar anatomical, physiological and embryological characteristics with the cerebral vasculature.[307;308] Quantitative measurement of retinal microvascular changes is now possible using reproducible, computer-assisted methods to examine retinal photographs.[8] Population-based studies further showed that subtle retinal vascular calibre changes (particularly narrower arterioles and wider venules) independently predicted stroke and stroke death.[4-7] Thus, studying the association of GI and retinal microvascular changes may provide insights into potential effects of postprandial blood glucose response on the microvasculature.

We investigated the associations of GI with stroke-related mortality and retinal microvascular calibre in an older Australian cohort. We aimed to test the hypothesis that

retinal microvascular changes may partly explain the reported association between high GI foods and stroke mortality. We also examined associations with dietary cereal fibre (CF), given its strong inter-relationship with GI.

Methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision, common eye diseases and other health outcomes in an urban, predominantly Caucasian population aged 49 years or older. The 1992-4 baseline study examined 3654 eligible residents of two postcodes of the Blue Mountains region, west of Sydney, Australia (82.4% response). Subsequent 5- and 10-year examinations of this cohort were conducted.[211;237] The study adhered to the Helsinki Declaration recommendations and was approved by the Sydney West Area Health Service Human Research Ethics Committee. Written, informed consent was obtained from all participants.

Retinal Photography

Detailed methods of grading the calibre of retinal arterioles and venules are described elsewhere.[22] In brief, at the baseline examination, 30° photographs of the macular, optic disc and other retinal fields of both eyes were taken, after pupil dilation, using a Zeiss FF3 fundus camera (Zeiss, Oberkochen, Germany). We used methods developed by the University of Wisconsin-Madison,[233] to measure the internal calibre of retinal arterioles and venules from digitized photographs. These were then summarised using established formulas,[28] that account for branching patterns and combine individual vessel calibres into summary indices, reflecting the mean arteriolar and venular calibres, respectively, of each eye.

Dietary Data

A standardised interview and examination was performed and participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular, from a Willett questionnaire[213] incorporating a nine-category frequency scale and standard portion size estimates. This FFQ had reasonable concurrent validity when validated against 4-day weighed food records collected on three occasions in one year.[214] The validation yielded an energy-adjusted Spearman coefficient of 0.82 between self-reported and weighed food records, and correctly classified 85% to within one quintile difference for dietary fibre. The corresponding coefficient for GI was 0.57 and correct classification of subjects to within one quintile difference for GI was 74%.[298] Dietary intakes were estimated using Australian Tables of Food Composition (NUTTAB 90),[216] and published GI values using the glucose=100 scale.[220] Additional GI data were obtained from the Sydney University GI Research Service (SUGiRS) online database (www.glycemicindex.com). In total, 88.9% of GI values were obtained from published values and 11.1% were interpolated from similar food items. The consumption of breakfast cereals, collected in the FFQ, was used to enhance accuracy of the GI calculations.

An overall GI value for each participant's diet was calculated by summating the weighted GI of individual foods in the diet. The weighting was proportional to the contribution of individual foods to total carbohydrate intake. We also extracted data on total fibre intake as well as the fibre contribution from cereals, vegetables and fruits.

The FFQ was attempted and returned by 3267 participants at baseline (89.4%), with 2897 (88.7% of those attempting the FFQ, 79.3% of total participants) having sufficiently complete and plausible FFQ data for analysis. Subjects were excluded when more than 12

FFQ questions were missing, if an entire page was blank, or if daily energy intakes were <2500 kJ or >18,000 kJ.[187;214]

Demographic, lifestyle and dietary variables

The interview included questions about past medical history, including physician-diagnosed history of stroke and myocardial infarction, and lifestyle factors such as smoking. Higher educational achievement was defined as attainment of educational qualifications (certificate, diploma or degree) after secondary schooling. A single measure of systolic blood pressure (SBP) and diastolic blood pressure (DBP) using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Body-mass index (BMI) was calculated as weight (kg)/height (m)^2. Fasting blood samples were processed the same day for: haemoglobin, white cell and platelet counts, glucose, total cholesterol, triglycerides, high density lipoprotein-cholesterol and fibrinogen levels by the Institute of Clinical Pathology, Westmead Hospital.

Stroke mortality

Mortality data since baseline (13 years) were obtained via data linkage with the Australian National Death Index (NDI) in December 2005. The sensitivity and specificity of Australian NDI data has been estimated to be 93.7% and 100% for all-cause deaths, respectively, and 92.5% and 89.6%, respectively, for cardiovascular deaths.[227;228] Stroke deaths (thrombotic, hemorrhagic) included the following ICD-9 codes (430.0-438.9) and ICD-10 codes (I60.0-I69.9) when listed as any cause of death. No validity data on stroke-related deaths were previously reported.

Statistical Methods

Statistical analyses were performed using Statistical Analysis System Version 9.1 (SAS Institute, Cary, NC). The dietary data and retinal vessel calibre data are cross-sectional, while the mortality data are longitudinal.

Subject intakes were divided into tertiles by their mean dietary GI or fibre intake. Dietary GI and fibre variables were adjusted for total energy intake using the Willett residual method.[241] This method was also used to assess the effect of nutritional variables on retinal arteriolar or venular calibre, independent of fellow vessel influences: venule-adjusted arteriolar calibre was defined using linear regression, with venular calibre as the independent variable and arteriolar calibre the dependent variable, by calculating residuals, and adding these to the expected mean arteriolar calibre. Arteriole-adjusted venular calibre was similarly defined. The resulting adjusted calibre variables represent the non-shared variance of each vessel measurement, respectively.[11;12;309]

Cox proportional hazards regression was used to assess hazard ratios (HR) with 95% confidence intervals (CI) for tertile of mean GI or CF consumption on 13-year stroke-related mortality, after adjusting for age, gender, SBP and DBP, BMI, smoking status, educational qualifications, fair or poor self-rated health, history of myocardial infarction and stroke, and presence of diabetes. The proportional hazards assumption was tested for GI or CF variables with stroke mortality and no violations were detected. Participants lost to follow-up were treated as non-deaths.

To determine the individual and joint effects of GI and CF on the risk of stroke, we stratified the population into three groups by unhealthy versus healthy dietary intakes of these two measures. First, persons in both the lowest tertile of GI and the highest tertile of CF were

considered healthy by both measures ('both healthy'). Second, persons in either the lowest tertile of GI or the highest tertile of CF, but not both, were considered healthy in only one measure ('either healthy'). Third, persons positioned in both the highest tertile of GI and the lowest tertile of CF were considered to have 'both unhealthy' categories. We assessed the HR of stroke for the 'both unhealthy' and 'either healthy' categories compared with the 'both healthy' category. The remaining three categories of persons were omitted from this analysis.

To investigate whether retinal venular calibre is an intermediate marker on the pathway between GI, CF and stroke risk, we included retinal venular calibre in the Cox regression models to assess whether the effect size was attenuated. We also evaluated synergy using the Rothman synergy index[310] to determine if the joint effects from GI and CF on the risk of stroke death or wider retinal venular calibre exceeded the sum of effects from each factor alone.

$$S_{ab} = \frac{(RR_{ab}-1)}{(RR_a + RR_b)-2}$$

RR_{ab} is the relative risk of the joint exposure group, RR_a and RR_b are relative risks for exposure to GI or CF, respectively. The synergy index represents the ratio of increased risk due to joint exposure (with synergistic effect) to the sum of increased risks due to each exposure alone.

The mean venule-adjusted arteriolar calibre and arteriole-adjusted venular calibre for GI or CF tertile was assessed using ANCOVA. The lowest tertile of GI and highest tertile of fibre consumption were the reference categories. Finally, we used logistic models to assess interactions between fibre consumption and GI in their effects on the retinal microvasculature, using the widest venular quintile as the outcome variable.

Three ANCOVA models were constructed: Model 1 adjusted for age, gender, SBP and DBP, BMI, smoking, educational qualifications, fair or poor self-rated health, diabetes mellitus, history of coronary heart disease and total vegetable, saturated fat and fish consumption. Model 2 was adjusted for variables in Model 1, plus the nutrient variables, vitamins C and E, beta-carotene, zinc and folate replacing the vegetable variable. Model 3 also adjusted for white cell count, haemoglobin and fibrinogen, when the dependent variable was mean venular calibre.[51;247;311;312]

Results

Of the 3654 baseline participants, 2897 participants with FFQs sufficiently complete and plausible were included for analysis. For the retinal vessel analysis, a further 185 participants were excluded due to missing retinal photographs or poor photographic images precluding vessel measurement, or with retinal diseases confounding measurement of retinal vessel calibre. Participants without usable FFQ data were more likely to be older (mean age 69.3 years vs. 65.3 years), or current smokers (17.7% vs. 14.2%) than those with usable FFQs. Among those completing the FFQ mean venule-adjusted arteriolar calibre was 187.3 microns and mean arteriole-adjusted venular calibre was 225.0 microns.

Over the 13 years, a total of 1297 participants had died (35.5% of original cohort) by December 2005, with 139 of these recorded as stroke-related deaths (3.8%). After accounting for persons with available FFQ data, 95 stroke-related deaths (3.5%) are included in this report.

Table 3.2.6 demonstrates the baseline characteristics of the population by GI tertiles. Male gender, fair or poor self-rated health, educational qualifications, white cell count, current

smoking and the consumption of vegetables, fish and several nutrients, differed between GI strata.

Table 3.2.7 demonstrates that higher mean dietary GI and lower CF consumption at baseline was associated with greater 13-year stroke-related mortality. After adjusting for age, gender, SBP and DBP, BMI, smoking status, educational qualifications, fair or poor self-rated health, history of myocardial infarction and stroke, and presence of diabetes, the HR of stroke-related death for persons with diets in the highest tertile of GI was 1.91 (95% CI 1.01 to 3.47) compared to those with diet in the two lower tertiles. The HR for the lowest tertile of CF consumption was 2.13, (95% CI 1.19 to 3.80) compared to the two higher tertiles. We found no relationship between total, vegetable or fruit fibre and risk of stroke-related death.

Joint effects of GI and CF on stroke-related death is shown in **Table 3.2.7**. The group with unhealthy diet in either category (either highest tertile of GI or lowest tertile of CF) had a near doubling of risk for stroke death compared to the group with healthy diet in both categories. The group with unhealthy diet in both categories had a 5-fold increased risk for stroke death (**Table 3.2.7**). The synergy index was 2, suggesting a substantial excess risk of stroke death attributable to joint exposure to both high GI and low CF diet.

We found no relationship between total carbohydrate consumption (excluding nondigestible fibre), glycemic load and stroke mortality (data not shown). There was also no relationship demonstrated between GI (T3 vs. remainder, HR, 0.91, 95% CI, 0.70-1.78), CF (T1 vs. remainder, HR, 0.94, 95% CI, 0.73-1.22) and the 13-year incidence of coronary heart disease mortality, suggesting some specificity of the observed associations with stroke-related death

(Table 3.2.7). However, we also found a higher risk of all-cause mortality in persons with both a high GI and low CF diet (HR, 1.48, 95% CI, 1.11-1.98).

Table 3.2.8 shows the mean arteriolar and venular calibre by mean dietary GI, and CF consumption, after adjusting for multiple potential confounding variables. Higher mean dietary GI was associated with narrower mean arteriolar calibre (p trend = 0.22), but wider mean venular calibre (p for trend = 0.01). Lower CF consumption was associated with significantly narrower arteriolar calibre (p trend = 0.002) and wider venular calibre ($p<0.001$). These associations persisted after replacing vegetable consumption with micronutrients in Model 2, or after further control for haemoglobin and fibrinogen in Model 3. Stratifying by the presence of hypertension or diabetes did not alter these relationships.

We found a statistical interaction between the effects of mean dietary GI and CF on venular calibre (p interaction = 0.002) (**Table 3.2.9**). Participants with both a high GI and low CF diet had two-fold greater odds of being in the widest category of retinal venular calibre. A synergy index of 1.50 suggested a greater effect of these two factors on wider venular calibre, when jointly present.

After adjusting for retinal venular calibre, the higher stroke mortality risk associated with high GI was reduced in magnitude and became non-significant (HR fell from 1.91 to 1.45). In contrast, the relationship between CF and stroke-related death persisted with similar magnitude (HR 2.13 vs 2.46, **Table 3.2.10**).

Discussion

Only a few studies have investigated the relationships between dietary GI[299-301], CF content and risk of stroke.[37;313-316] The underlying pathways of these associations have not previously been examined in detail. In this older population-based cohort, we showed that either a high GI or low CF diet predicted a doubling of the long-term risk of stroke-related death. These two dietary factors also appeared to act synergistically to increase stroke risk more than 5-fold. The inclusion of retinal venular calibre in our model resulted in a 50% attenuation of the risk of stroke-related mortality from higher GI diets, indicating that 50% of the stroke death risk associated with high GI could be explained by the association with wider retinal venular calibre. This suggests a possible mechanism by which dietary parameters affecting post-prandial glycaemia could influence stroke-related mortality. In contrast, excess stroke mortality associated with low dietary CF appeared independent of retinal venular calibre.

Retinal venular widening has been identified as a structural microvascular sign predicting higher risk of stroke and other cardiovascular diseases,[4;8-13]. We previously documented a non-significant relationship between larger venular diameter (>255.5 microns) and incident stroke-related death (HR, 1.75, 95% CI, 0.75–4.07) in persons younger than 75 years.[13] Our current report provides further evidence in support of the concept that the microvasculature is a potential pathway by which high GI diet impacts adversely on stroke risk.[4-7] Potentially deleterious cerebral effects of post-prandial glucose could thus operate through the cerebral microvasculature, assuming that these retinal vessel signs parallel changes in cerebral vessels.

Several potential biological mechanisms could be operating at the microvascular level through which higher GI diets could mediate stroke risk. It has been suggested that the

endothelial dysfunction preceding stroke may be mediated by the formation and collection of advanced glycation end products in vessel walls, producing vascular damage such as increased vascular permeability.[302] The vascular endothelium is particularly susceptible to high levels of postprandial glycaemia as endothelial cells are unable to regulate glucose transport across the cell membrane.[305;317] Inflammation or reduced antioxidant capacity from hyperglycemia could also mediate endothelial dysfunction.[304;305]

Recent studies found no relationship between high GI diets and cardiovascular mortality, similar to our findings.[300;318;319] Levitan et al reported a relationship between hemorrhagic stroke and high GI. This could not be confirmed in our study because of the relatively small number of participants with hemorrhagic stroke on ICD classification (n=9).

We showed that diets low in CF were associated with adverse changes not only in retinal venular calibre but also in retinal arteriolar calibre. Many studies have shown that consumption of wholegrain cereals (CF being an important constituent) is inversely associated with cardiovascular disease,[37] and results in modest blood pressure reductions.[38;39] However, we did not observe any attenuation of the magnitude of stroke risk, after accounting for retinal venular calibre in our statistical models, suggesting that its associations with risk of stroke-related death and with microvascular changes are independent.

The different pattern of diet relationships between arteriolar and venular calibre may represent different pathogenic processes affecting arterioles and venules. Wider venules, for example, have been observed in association with inflammatory factors and endothelial dysfunction,[51;247;311;312] in contrast to arteriolar calibre, which is principally affected by hypertension.[8;51]

Strengths of this study include its well-defined urban population, use of a validated food questionnaire to collect dietary information and detailed questionnaires that permitted careful assessment of potential confounding variables. The retinal vessel grading was masked, with good intra-grader reliability.[22;28] Measurement error in the assessment of dietary variables is likely to be non-differential as the dietary factors were collected long before stroke death events occurred. Participants may have altered their fibre consumption after diagnosis with hypertension or cardiovascular disease, but they would have been less likely to have altered the GI of their diets, as there was little publicity about the potential benefits of lower GI diet during the period 1992-4. An important limitation of our study is the cross-sectional nature of the associations of GI and vessel calibre. Another limitation of our study is the use of a single blood pressure measurement, which may not accurately reflect a participant's 'usual' blood pressure over time.

Despite our best efforts to control for socio-economic and lifestyle variables, incomplete control for confounding from unmeasured social factors may have occurred. Residual confounding, however, seems unlikely to have had a major influence on our findings given their internal consistency: i.e. different patterns of dietary associations were observed (e.g. CF, but not total or fruit fibre, was associated with stroke), that would be difficult to explain by confounding from unmeasured lifestyle factors. Finally the relatively low sensitivity and specificity of death certificate data could have tended to misclassify some stroke deaths, but this would likely only result in an underestimation of the association.

It should be recognised that the retinal vessel calibre differences observed between the lowest and highest categories of dietary GI and CF were modest (the between-person variation was 16.8 microns in arteriolar calibre and 16.3 microns in venular calibre). It has been shown, however, that even such a small reduction in retinal arteriolar calibre can be

associated with moderate changes in blood pressure: for example, each 10mmHg increase in systolic blood pressure was associated with a 1.1 micron reduction in arteriolar calibre.[51] Confirmation of our results in similar population-based studies such as those in the US and the Netherlands would strengthen these findings.[4-8;287] Experimental studies examining microvascular structure and function may also help to elucidate mechanisms underling our findings.

In conclusion, we showed that diets with high GI and low CF content predicted greater stroke mortality. These diets were also associated with wider retinal venular calibre, an intermediate microvascular marker of stroke. The increased risk of stroke mortality associated with high GI diets was attenuated by 50% after accounting for variations in retinal venular calibre. Although microvascular changes are known to precede cardiovascular events, our findings indicate that the deleterious cerebrovascular effects from high GI diets could operate partly by anatomical effects on the cerebral microvasculature.

Table 3.2.6. Characteristics of participants at the baseline Blue Mountains Eye Study examination by glycemic index tertiles

Characteristics	Glycemic Index tertile 1	Glycemic Index tertile 2	Glycemic Index tertile 3
Mean age (yrs)	64.8	65.6	65.7
Male gender (%)	30.6	44.6	56.7
Fair or poor self-rated health (%)*	20.1	21.5	26.1
Educational qualifications (%)†	55.4	50.4	43.8
Systolic blood pressure (mmHg)	146	145	147
Diastolic blood pressure (mmHg)	83	83	84
Body-mass index	26.3	26.1	26
Fasting serum cholesterol (mmol/L)	6.1	6.2	6.0
White cell count ($x10^9$)	6.3	6.5	6.7
Current smoker (%)	8.9	12.9	21
Presence of diabetes mellitus (%)	8.9	6.1	6.6
History of coronary heart disease (%)	7.6	8.8	8.4
History of stroke (%)	4.7	5.2	3.9
Consumption of:			
vegetables (grams/day)	452.4	440.2	432.8
fish (grams/day)	30.7	28.7	22.5
Dietary intake of:‡			
saturated fat (grams/day)	29.0	30.4	29.7
cereal fibre (grams/day)	8.5	7.7	5.9
vitamin E (mg/day)	54.1	37.7	27.2
vitamin C (mg/day)	416.3	340.2	260.6
betacarotene (µg /day)	8135.8	7484.5	6505.4
zinc (mg/day)	12.3	11.7	11.1
folate (µg /day)	439.9	400.9	390.2

*Self-rated health described as "fair" or "poor" compared with "excellent" or "good"

†Any post secondary school qualification (inc. trade certificate, diploma, degree)

‡Antioxidant amounts include both diet (energy-adjusted) and supplements

Table 3.2.7. Hazard ratios (95% confidence intervals, CI) of 13-year stroke-related and coronary heart disease-related death, by tertiles of glycemic index and cereal fibre consumption*

Variable		Median	No. at risk	n (deaths)	Stroke related death	Coronary-heart disease related death
					RR (CI)	RR (CI)
Mean dietary glycemic index†	Tertile 1	52.4	965	19	1.0	1.0
	Tertile 2	56.5	966	41	1.75 (0.94-3.25)	0.80 (0.61-1.04)
	Tertile 3 (highest)	60.6	966	35	1.91 (1.01-3.47)	0.91 (0.70-1.78)
	P trend				0.04	0.54
Cereal fibre consumption†	Tertile 3	11.0	981	37	1.0	1.0
	Tertile 2	6.5	966	36	2.03 (1.13-3.61)	1.22 (0.95-1.58)
	Tertile 1 (lowest)	3.0	950	22	2.13 (1.19-3.80)	0.94 (0.73-1.22)
	P trend				0.02	0.65
Glycemic index and low cereal fibre jointly‡§	Both healthy		394	8	1.0	1.0
	Either healthy		1431	53	1.88 (0.83-4.22)	1.11 (0.78-1.57)
	Both unhealthy		428	19	5.06 (1.67-15.22)	1.07 (0.68-1.67)
	Rest of group		549	15	1.83 (0.83-4.02)	1.03 (0.74-1.45)

*Adjusted for continuous age, gender, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication use, body-mass index, smoking status, educational qualifications, fair or poor self-rated health, history of myocardial infarction and stroke, and presence of diabetes

†Energy-adjusted

‡Both healthy=lowest tertile glycemic index and highest tertile cereal fibre

Either healthy=either lowest tertile of glycemic index or highest tertile of cereal fibre, but not both

Both unhealthy=highest tertile of glycemic index and lowest tertile of cereal fibre

Rest of group=includes persons omitted from above categories

§Smaller number of deaths due to incomplete overlap of categories

Table 3.2.8. Mean retinal arteriolar and venular calibre (95% confidence intervals) by tertiles of glycemic index and cereal fibre (cross-sectional analysis). *

Variable	Median	Mean retinal arteriolar calibre (μm)	Mean retinal venular calibre (μm)
Mean glycemic index†			
Tertile 1	52.5	187.8 (186.8-188.7)	224.1 (223.0-225.2)
Tertile 2	56.5	187.2 (186.2-188.1)	224.8 (223.8-225.9)
Tertile 3 (highest)	60.6	186.9 (186.0-187.9)	226.1 (225.0-227.2)
p for trend		0.22	0.01
Cereal fibre consumption †			
Tertile 3	11.0	188.4 (187.5-189.4)	223.4 (222.3-224.4)
Tertile 2	6.5	187.1 (186.1-188.0)	225.4 (224.4-226.5)
Tertile 1 (lowest)	3.3	186.3 (185.4-187.3)	226.2 (225.1-227.3)
p for trend		0.002	<0.001

*Adjusted for continuous age, gender, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication use, body-mass index, smoking status, educational qualifications, fair or poor self-rated health, history of myocardial infarction and stroke, and presence of diabetes

†Energy-adjusted

Table 3.2.9. Synergistic effect *of high glycemic index and low cereal fibre consumption on the likelihood of having wider retinal venular calibre (defined as the widest quintile)

	Mean glycemic index (GI) tertile		
Cereal fibre tertile	Low GI (Tertile 1)	Medium GI (Tertile 2)	High GI (Tertile 3)
High fibre (Tertile 3)	1.00	1.43 (0.91-2.24)	1.49 (0.97-2.28)
Medium fibre (Tertile 2)	1.01 (0.65-1.58)	1.30 (0.85-1.99)	2.00 (1.33-3.03)
Low fibre (Tertile 1)	1.32 (0.81-2.15)	1.90 (1.32-2.79)	2.22 (1.38-3.18)

*Odds ratios (with 95% confidence intervals) adjusted for continuous age, gender, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication use, body-mass index, smoking status, educational qualifications, fair or poor self-rated health, history of myocardial infarction and stroke, and presence of diabetes. Interaction between dietary glycemic index and cereal fibre, $p = 0.002$.

Table 3.2.10. Multivariate* adjusted hazard ratio (95% confidence interval) of stroke-related death for higher glycemic index or lower cereal fibre consumption, after adjustment for retinal venular or arteriolar diameter.

Variable	Stroke related death RR (95% CI)	Stroke related death RR (95% CI), with inclusion of retinal venular diameter in model†	% reduction in excess risk due to retinal venular diameter‡
Higher mean glycemic index	1.91 (0.95-3.47)	1.45 (0.75-2.93)	51%
Lower cereal fibre consumption	2.13 (1.19-3.80)	2.46 (1.28-4.73)	NA

*Adjusted for continuous age, gender, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication use, body-mass index, smoking status, educational qualifications, fair or poor self-rated health, history of myocardial infarction and stroke, and presence of diabetes

†Inclusion of arteriolar diameter as well, in model for cereal fibre

‡% reduction in excess risk defined by the formula: $(r_a - r_b) / (r_a - 1)$, where r_a is the RR of stroke for increasing glycemic index adjusted for other variables (reference) but not adjusting for retinal venular diameter and r_b is the RR after additional adjustment for retinal venular diameter

Chapter 7: Is Diet related to the prominent retinal arteriolar light reflex sign?

Abstract

Purpose: We aimed to assess the association between diet and the enhanced retinal arteriolar light reflex sign.

Design and participants: Population-based, cross-sectional study comprising 3654 participants (82.4% response) aged 49+ years from the Blue Mountains region, Australia.

Methods: Retinal photographs of participants were graded for presence and severity of the enhanced arteriolar wall light reflex, by comparison with standard photographs. Baseline dietary information was collected using a food frequency questionnaire. Associations of the enhanced reflex with dietary factors (subject-specific) were assessed using logistic regression after adjusting for age, gender, body-mass index, smoking status, presence of cataract, serum glucose and total dietary consumption of fats, protein and carbohydrates and presented as odds ratio (OR).

Main Outcome Measures: Mild and marked levels of the enhanced arteriolar wall reflex.

Results: Of 3654 baseline participants, 3520 had gradable photographs. After excluding persons with cataract, increasing consumption of vitamin C (OR for 3rd tertile 1.83, 95% confidence interval, CI, 0.91 to 3.67, p_{trend}=0.04) was associated with the markedly enhanced retinal arteriolar wall reflex. There were no associations with dietary intake and the mildly enhanced arteriolar light reflex. Participants with greater than the median level intake of all micronutrients had a 4-fold higher odds of having the marked arteriolar wall reflex, although the small number of persons in the markedly enhanced group limited the study power.

Conclusions: This is the first study to examine an individual's diet and its relationship to an enhanced reflex on their retinal arterioles. Our findings suggest that some healthy dietary components are associated with a marked level of this sign. These results are in contrast with previous theories asserting the strong relationship of this sign with arteriosclerosis. Studies in other populations would assist in confirmation of these results.

Introduction

The ophthalmoscopic appearance of a centralised light reflection from the surface of retinal arterioles is termed the "arteriolar light reflex."[25] Alterations to the light reflex of the retinal arteriolar wall, specifically widening of the reflex zone at the centre of the arteriole and an increased reflex intensity, have been incorporated into diverse classification schemes describing hypertensive retinopathy for many years.[8;262;263] We previously reported the prevalence and associations with the enhanced retinal arteriolar light reflex in a population-based sample of older Australians aged 49+ years, and found that only severe level of the enhanced light reflex of retinal arterioles was associated with elevated blood pressure.[25] Although this sign has traditionally been considered indicative of sclerosis of small arteriolar wall, its clinical implication and utility is unclear, with a few associated factors being identified.[25]

The retinal imaging and computer-assistant image assessment methods enable quantitative and qualitative assessments of microvascular signs with improved reliability, allowing measurement of subtle variations in arteriolar or venular calibre. We previously assessed the link between dietary factors and generalised arteriolar narrowing, and documented a beneficial effect on the microvasculature of consuming a diet high in fish or low in glycemic index.[320;321]

We postulated that a poor diet might be associated with enhanced arteriolar wall reflex, if this sign is a true indication of arteriosclerosis. In order to further characterise this well-known and easily recognised sign of the retina, we aimed to assess the relationship between mild and moderate levels of this sign with dietary micronutrients that are considered beneficial to the eye and the heart.

Methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision, common eye diseases and other health outcomes in an urban predominantly Caucasian population aged 49 years or older. The baseline study, conducted during 1992-4, examined 3654 eligible potential participants living in two postcode areas in the Blue Mountains, west of Sydney, Australia (82.4% response). This study was conducted in accordance with recommendations of the Declaration of Helsinki and was approved by the Western Sydney Area Human Ethics Committee. Written, informed consent was obtained from all participants. Details of recruitment methods were previously described.[211;237].

Retinal Grading

At baseline examinations, 30° stereoscopic retinal photographs of both eyes were taken of the macula and optic disc, plus non-stereoscopic photographs of four other retinal fields (temporal, nasal, and both upper and lower vascular arcades), after pupil dilation, using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany). Retinal photographs of both eyes were assessed by graders, masked to participant characteristics, for presence of none, questionable, mild or marked enhancement of the retinal arteriolar light reflex, using a similar grading technique described for other focal retinal vessel abnormalities.[25;233;272] Briefly, presence of mildly or markedly enhanced light reflex was graded from 35mm slides of both eyes using a light box (Kelvin rating approximately 6200 degrees) and Donaldson stereoscopic viewer with x5 magnification. Photographic standards were selected by a retinal specialist (PM) from the slide set of the Modified Airlie House Classification of Diabetic Retinopathy and BMES participant photographs. Mild enhancement of the retinal arteriolar light reflex was defined as the presence of a central reflex with a sharp margin, occupying at

least one-third the width of the arterioles and consistently present (without interruption) over at least two-thirds the length of the arteriolar sector visible in the field. The width of the reflex was compared with that in the mildly enhanced retinal arteriolar light reflex photographic standard. Marked enhancement of the retinal arteriolar light reflex was defined as presence of a central reflex whose width was greater than 50% of the arteriolar wall width, irrespective of its intensity but consistently present over at least two-thirds the length of the arteriolar sector visible in the field. The grading of markedly enhanced arteriolar light reflex was required to be similar to or more obvious than the markedly enhanced retinal light reflex photographic standard. Any enhancement of the arteriolar wall reflex was defined to include both mild and marked levels. Although methods used for grading of other focal vascular abnormalities have excluded the zone within one half disc diameter circumference from the optic disc, grading for the presence of an enhanced light reflex did not exclude this zone due to the requirement to assess consistency of the reflex along the whole course of arterioles. Grades were adjudicated by a senior researcher, and for equivocal or difficult cases, by a retinal specialist (PM). The intra-grader reliability was assessed, with simple kappa statistic of 0.75 and weighted kappa statistic of 0.84.

Dietary Data

As part of the interview and examination, participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular from a Willett questionnaire,[213] that incorporated a nine-category frequency scale and standard portion size estimates. The FFQ had reasonable concurrent validity[214] when validated against 4-day weighed food records collected on three occasions in one year (n=79). Correlation coefficients (r) comparing the FFQ and weighed food records were over 0.50 for all nutrients except protein (r=0.16), vitamin A (r=0.32) and iron(r=0.37). For fats, the FFQ was found to show moderate to good agreement for ranking individuals according to their fat intakes,

yielding correlation coefficients between 0.4-0.7, and correctly classifying over 70 percent of people within one quintile for all types of fats.[214] Questions were asked about specific foods and supplements. Dietary supplements were coded using supplement information reported in the Australian Register of the Therapeutic Goods Administration. Dietary intakes were estimated using Australian Tables of Food Composition (NUTTAB90),[216] and a fatty acid supplement database.[218;219]

We extracted information for the following nutrients; vitamin C, vitamin E, zinc, beta-carotene, lutein and zeaxanthin and the B-group vitamins, thiamin, niacin, riboflavin and vitamin B12. We also extracted information for one of the primary food sources of these vitamins, fruits and vegetables. These micronutrients were analysed as they were the most frequent nutrients in the literature to be assessed in relation to the common eye conditions of macular degeneration and cataract.[322]

Demographic, lifestyle and dietary variables

A standardised interview and examination was performed on all participants during 1992-94. The interview and examination was used to collect information on demographic and health-related information such as physician-diagnosed history of stroke or myocardial infarction and lifestyle factors such as smoking. Examination incorporated assessment of blood pressure, weight and height. A single measure of systolic and diastolic blood pressure using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Body mass index (BMI) was calculated as weight (kg) /height $(m)^2$. Fasting blood samples were processed on the same day for total cholesterol and glucose by the Institute of Clinical Pathology, Westmead Hospital. Diabetes was defined either from past history of diabetes and current diabetes treatment, or from fasting plasma glucose levels $\geq$7.0mmol/L at examination, using the World Health Organization diabetes classification.[224]

Statistical Analysis

Statistical analyses were performed using Statistical Analysis Software (version 9, SAS Institute, Cary, NC). Analyses were conducted using person-specific data, and mildly or markedly enhanced retinal arteriolar light reflex was classified based on the worse eye of the person. We examined the cross-sectional association between the consumption of micronutrients and primary sources of micronutrients, fruits and vegetables, and the presence of mild or marked enhanced retinal arteriolar light reflex at baseline. Logistic regression models were constructed to assess associations between dietary factors and the arteriolar light reflex, controlling for age, gender, body-mass index, smoking status, presence of cataract, serum glucose and total dietary consumption of fat, protein and carbohydrates. These covariates were previously found to be significantly associated with enhanced arteriolar wall reflex.[25] Micronutrient variables were adjusted for total energy intake using the Willett residual method.[241] Subject intakes were divided into tertiles for micronutrient and food variables. Odds ratios (OR), 95% confidence intervals (CI) and adjusted p values for trend are presented.

We also conducted an analysis using three categories of combined micronutrient consumption, defined using the median energy-adjusted daily intake per nutrient as the cutoff. Persons with above-median intake of all nutrients were classified as having a high intake and persons with below-median intake of all nutrients were classified as having low intake. All other persons were included in the reference or middle group. Individual micronutrients assimilated in this way and their corresponding cutoff values are; 201.4mg for vitamin C, 8.1mg for vitamin E, 6702.4µg for beta- carotene, 11.7mg for zinc, 730.1mg for lutein and zeaxanthin, 2.42mg for thiamin, 5.7mg for riboflavin, 23.5mg for niacin, and 5.4mg for vitamin B12.

Since our previous study showed a strong inverse relationship between the presence of cataract and the mildly enhanced retinal arteriolar reflex,[25] we analysed data after excluding participants with cataract (findings present in tables).

Results

Of 3654 baseline BMES participants, 3520 (96.3%) had photographs gradable in at least one eye and data available for this study, after excluding 40 persons with missing blood pressure data and 134 persons with missing photographs (overlap in 28 persons). The retinal arteriolar wall reflex was graded as mildly enhanced in 1052 participants (29.9%) and markedly enhanced in 105 participants (3.0%). Combining both eyes, 460 persons (13.1%) had mildly enhanced light reflex bilaterally and only 8 persons (0.2%) had markedly enhanced light reflex bilaterally.

Table 3.2.11 shows the baseline characteristics of the study sample based on relevant characteristics known to be associated with the enhanced retinal arteriolar reflex. Age, presence of hypertension, body-mass index, current smoking, serum cholesterol and presence of cataract were differentially distributed between those with and without the mild or markedly enhanced arteriolar light reflex.

Table 3.2.12 shows dietary micronutrients associated with presence of mildly or markedly enhanced retinal arteriolar light reflex, after multivariable adjustment. Increasing tertile of consumption of vitamin C (odds ratio for 3rd tertile, OR, 1.83, 95% confidence interval, CI, 0.91 to 3.67, p_{trend}=0.04) was associated with significantly higher odds of a markedly enhanced light reflex. There were no associations demonstrated for the mildly enhanced arteriolar light reflex and dietary micronutrient consumption.

The multivariable-adjusted associations between B-group vitamins and the two levels of the enhanced arteriolar reflex (Table 3.2.13) do not show any significant trends.

Table 3.2.14 demonstrates the relationship between a primary food source of the micronutrients, vitamin C, vitamin E, beta-carotene and lutein and zeaxanthin and the mildly and markedly enhanced retinal arteriolar light reflex. There appears to be a decreased prevalence of the mildly enhanced retinal arteriolar light reflex with increasing consumption of vegetables (3^{rd} tertile, OR, 0.76, 95% CI, 0.59 to 0.99, p_{trend}=0.07). There was no demonstrated association between both fruits and vegetables and the markedly enhanced retinal arteriolar light reflex.

Table 3.2.15 shows the effect of the combined consumption of micronutrients and the prevalence of the enhanced arteriolar light reflex. For persons with above median consumption level of vitamin C, vitamin E, beta-carotene, zinc, lutein and zeaxanthin, and B-group vitamins, thiamin, riboflavin, niacin and vitamin B12, there was a four-fold higher odds of having the markedly enhanced arteriolar wall reflex (OR, 4.74, 95% CI, 1.98 to 11.36), however, the number of cases in the exposed group was only 9. In those with below median consumption level of these micronutrients, there was no association with the enhanced reflex (OR, 1.55, 95% CI, 0.54 to 4.44).

Results from the entire sample (including participants with cataract) were similar for the markedly enhanced retinal arteriolar reflex group. For the mildly enhanced retinal arteriolar reflex group, a non-significant, reverse association between thiamine consumption and presence of the mild reflex was evident (data not shown).

Discussion

This is the first study to assess the relationship between diet and the enhanced retinal arteriolar wall light reflex. Our aim was to further understand and characterise and associations of this sign, given the long-held belief that this sign is strongly related to arteriosclerotic disease and hypertension. Though it cannot be disputed that the completely opaque, white or grayish 'ghost' vessels seen in end-stage retinal vascular disease represent obvious occlusive arteriosclerotic pathology, the clear yellow-white reflex seen in the central zone of a vessel wall width is not well-correlated in terms of anatomic evidence of pathologic changes in the vessel wall,[263;269] largely due to unavailability of histopathological specimens.

Among the nutritional factors assessed in this study, we found that only a single nutritient was positively associated with a higher prevalence of this sign: greater amounts of vitamin C intake from the diet was associated with a higher frequency of having the markedly enhanced retinal arteriolar wall light reflex. However, when we combined all the micronutrients individually assessed, we found that those persons with higher median consumption of all these micronutrients had an almost 4-fold increase in the odds of having a markedly enhanced retinal arteriolar light reflex. Due to the very few number of persons (9 out of 83) in our sample with the markedly enhanced arteriolar wall light reflex who were also in the upper median intake group, we cannot exclude the possibility of a chance finding. We could not document any associations between antioxidants intake and the mild level of this sign.

Since the enhanced arteriolar reflex is more prominent in persons without cataract, and consumption of vitamin C, vitamin E, riboflavin, niacin and other antioxidants is associated

with a lower incidence of cataract,[322] it may be that the relationship between the presence of the arteriolar light reflex and healthy aspects of diet is explained by persons with a healthy diet having a lower likelihood of cataract. However, our findings were evident after exclusion of persons with cataract. We previously found that hypertension was related to the markedly enhanced arteriolar light reflex. Persons with hypertension could have changed their diet in response to their diagnosis. This indication bias could be an alternative explanation of the current finding of high intake of vitamin C associated with the markedly enhanced arteriolar wall light reflex.

Brinchmann-Hansen et al, demonstrated that the retinal arteriolar reflex probably emanates from the column of densely packed erythrocytes coursing through the retinal microvasculature, viewed through the normally transparent blood vessel wall.[268;276;277;279] There is good evidence to show that low plasma levels of vitamin C can produce dyslipidaemia and subsequent vascular atherogenic changes.[323] Thus vitamin C can protect against atherosclerotic changes to the arteriolar wall.[323] It may be that a diet high in antioxidants leads to a more prominent reflex emanating from erythrocyte flow, observed through a more transparent arteriolar wall, however this hypothesis is purely speculative.

There are several weaknesses of the study. Firstly, the number of cases with the markedly enhanced reflex of the retinal arteriolar wall was small. The cross-sectional nature of this study can only generate research questions for future studies but not provide a conclusive link between diet components and the retinal arteriolar wall light reflex. Secondly, despite our best efforts to control for socioeconomic factors, incomplete control of important confounding factors from unmeasured lifestyle and social factors may exist. Thirdly, the use of a single blood pressure measurement may not accurately reflect a participant's 'usual'

blood pressure. Finally, our multiple statistical comparisons make it possible that some of the associations found were due to chance.

Strengths of this study include the fact that it was conducted in a well-defined urban population using an interviewer administered questionnaire, which collected many potential confounding variables. Manual grading of the retinal vessel lesions was rigorous, with good intra-grader reliability and was performed in a masked fashion. Since participants were unlikely to be aware of their retinal vessel diameters or any retinal vessel wall signs, differential measurement error in the assessment of dietary variables is improbable.

In conclusion, our study findings show that higher dietary consumption of vitamin C was associated with the presence of the markedly enhanced retinal arteriolar light reflex. The above median intake of a combination of antioxidant nutrients was associated with a significantly higher odds of possessing the light reflex. These associations are in contrast to previous ideas regarding the link between this sign and arteriosclerosis. Potential confounding effects, biases and chance findings need to be considered. Longitudinal studies will provide better information about the clinical implication of this sign and the association of diet with the sign.

Table 3.2.11. Baseline characteristics of the study sample (n=3520)

Characteristics*	Presence of Mildly Enhanced Arteriolar Reflex (n=1052)	Presence of Markedly Enhanced Arteriolar Reflex (n=105)	No Enhanced Arteriolar Reflex (n=2363)
Age (yrs)	63.4	64.4	66.7
Male gender (%)	43.4	54.1	43.9
Presence of hypertension (%)	43.1	46.0	60.1
Body-mass index	26.6	26.3	25.9
Current smoker (%)	17.9	16.2	14.2
Fasting serum glucose (mmol/L)	5.38	4.93	5.22
Fasting serum cholesterol (mmol/L)	6.08	6.37	6.00
Presence of any cataract (%)	23.1	24.3	36.3

*Data are expressed as means unless otherwise indicated

Table 3.2.12 - Multivariate adjusted* odds ratio (95% confidence interval) for association between presence of mild or marked enhanced arteriolar reflex and increasing tertile of micronutrients

Food Type			Mild	Marked
	No. at Risk	No. Affected (%)	OR (95% CI)†	OR (95% CI)†
Vitamin C (milligrams)				
Tertile 1	632	313	1	1
Tertile 2	626	312	0.98 (0.76-1.26)	1.10 (0.52-2.31)
Tertile 3	629	311	0.98 (0.76-1.27)	1.83 (0.91-3.67)
p for trend			0.78	0.04
Vitamin E (milligrams)				
Tertile 1	611	327	1	1
Tertile 2	650	294	1.03 (0.80-1.33)	0.82 (0.41-1.65)
Tertile 3	626	315	1.15 (0.89-1.49)	1.08 (0.57-2.08)
p for trend			0.49	0.79
Zinc (milligrams)				
Tertile 1	639	303	1	1
Tertile 2	627	310	1.00 (0.76-1.33)	1.06 (0.51-2.21)
Tertile 3	621	323	0.86 (0.60-1.23)	1.88 (0.34-2.27)
p for trend			0.30	0.51
Beta-carotene (micrograms)				
Tertile 1	620	315	1	1
Tertile 2	626	314	0.95 (0.73-1.22)	0.74 (0.38-1.44)
Tertile 3	641	307	0.87 (0.67-1.13)	0.74 (0.37-1.48)
p for trend			0.34	0.43
Lutein and zeaxanthin (micrograms)				
Tertile 1	633	310	1	1
Tertile 2	626	308	1.07 (0.84-1.38)	0.89 (0.44-1.79)
Tertile 3	628	318	0.88 (0.68-1.15)	1.26 (0.64-2.47)
p for trend			0.36	0.51

*Adjusted for age, gender, body-mass index, serum glucose, smoking status, presence of hypertension and cataract and total dietary fat, protein and carbohydrate consumption

†OR (95% CI) = odds ratio (95% confidence interval)

Table 3.2.13 - Multivariate adjusted* odds ratio (95% confidence interval) for association between presence of mild or marked enhanced arteriolar reflex and increasing tertile of B-vitamins

Food Type			Mild	Marked
	No. at Risk	No. Affected (%)	OR (95% CI)†	OR (95% CI)†
Thiamin (milligrams)				
Tertile 1	221	131	1	1
Tertile 2	236	116	0.79 (0.53-1.20)	1.20 (0.47-3.12)
Tertile 3	235	114	0.88 (0.59-1.31)	1.30 (0.51-3.26)
p for trend			0.47	0.68
Niacin (milligrams)				
Tertile 1	643	296	1	1
Tertile 2	615	324	1.09 (0.85-1.41)	1.17 (0.56-2.44)
Tertile 3	629	316	0.99 (0.76-1.30)	1.69(0.83-3.44)
p for trend			0.80	0.18
Riboflavin (milligrams)				
Tertile 1	619	319	1	1
Tertile 2	646	293	0.97 (0.76-1.25)	1.08 (0.55-2.12)
Tertile 3	622	324	0.88 (0.59-1.31)	1.11 (0.56-2.22)
p for trend			0.60	0.66
Vitamin B12 (milligrams)				
Tertile 1	640	289	1	1
Tertile 2	634	311	1.14 (0.88-1.48)	1.51 (0.72-3.15)
Tertile 3	613	336	1.28 (0.98-1.69)	1.66 (0.78-3.52)
p for trend			0.25	0.26

*Adjusted for age, gender, body-mass index, serum glucose, smoking status, presence of hypertension and cataract and total dietary fat, protein and carbohydrate consumption
†OR (95% CI) = odds ratio (95% confidence interval)

Table 3.2.14 - Multivariate adjusted* odds ratio (95% confidence interval) for association between presence of mild or marked enhanced arteriolar reflex and increasing tertile of fruits and vegetables

Foods			Mild	Marked
	No. at Risk	No. Affected (%)	OR (95% CI)†	OR (95% CI)†
Fruits (grams)				
Tertile 1	611	327	1	1
Tertile 2	627	319	1.01 (0.79-1.30)	0.78 (0.39-1.57)
Tertile 3	651	291	0.85 (0.65-1.12)	1.08 (0.54-2.16)
p for trend			0.25	0.99
Vegetables (grams)				
Tertile 1	611	327	1	1
Tertile 2	627	319	0.98 (0.76-1.25)	0.25 (0.11-0.59)
Tertile 3	651	291	0.76 (0.59-0.99)	0.91 (0.50-1.67)
p for trend			0.07	0.71

*Adjusted for age, gender, body-mass index, serum glucose, smoking status, presence of hypertension and cataract and total dietary fat, protein and carbohydrate consumption
†OR (95% CI) = odds ratio (95% confidence interval)

Table 3.2.15 - Multivariate adjusted* odds ratio (95% confidence interval) for association between presence of mild or marked enhanced arteriolar reflex and category of consumption of 8 micronutrients

	Lower median of antioxidant intake†	Middle (reference group)†	Upper median of antioxidant intake†
Mild			
No. at risk	586	1726	51
No. affected	248	781	23
OR (95% CI)‡	1.14 (0.73-1.77)	1	1.24 (0.69-2.25)
Marked			
No. at risk	834	2507	74
No. affected	27	69	9
OR (95% CI)‡	1.55 (0.54-4.44)	1	4.74 (1.98-11.36)

*Adjusted for age, gender, body-mass index, serum glucose, smoking status, presence of hypertension and cataract and total dietary fat, protein and carbohydrate consumption

†Definition of categories: the median energy-adjusted daily intake per nutrient was the cutoff, with persons with above-median intake of all nutrients classified as high intake and persons with below-median intake of all nutrients as low intake. All other persons were in the reference or middle group. Cutoff values were 201.4mg for vitamin C, 8.1mg for vitamin E, 6702.4µg for beta carotene, 11.7mg for zinc, 730.1mg for lutein and zeaxanthin, 2.42mg for thiamin, 5.7mg for riboflavin, 23.5mg for niacin, and 5.4mg for vitamin B12

‡OR (95% CI) = odds ratio (95% confidence interval)

Part 4

Diet and Age-Related Macular Degeneration

Chapter 8: Glycemic index and the 10 year incidence of age-related macular degeneration

Abstract

Background: Dietary parameters are known risk factors for age-related macular degeneration (AMD), the leading cause of visual loss among people 65 years and older. High glycemic index diets have been hypothesised as a risk factor for AMD, but prospective data are unavailable.

Objective: To examine the association between dietary glycemic index and the 10-year incidence of AMD.

Design: Population-based cohort study with 3,654 participants (49+ years) examined at baseline (1992-4), 2,335 re-examined after 5 years and 1,952 after 10 years. 10-year incident early and late AMD was graded from retinal photographs using the Wisconsin System. Baseline dietary information was collected using a food frequency questionnaire and the mean glycemic index was calculated using an Australian database.

Results: Over 10 years, 208 of 1810 persons (cumulative incidence 14.1%) developed early AMD. After adjusting for age, smoking, other risk factors and dietary constituents, higher mean dietary glycemic index was associated with an increased 10-year risk of early AMD (relative risk, RR, 1.77, 95% confidence interval, CI, 1.13–2.78, comparing 4^{th} to 1^{st} quartile, p_{trend}=0.03). Conversely, greater consumption of cereal fibre (RR 0.68, 95% CI 0.44-1.04, p_{trend}=0.05) and breads and cereals (predominantly lower glycemic index foods like oatmeal) (RR 0.67, 95% CI, 0.44-1.02, p_{trend}=0.03) was associated with a reduced risk of incident early AMD. No relationship was demonstrated with late AMD.

Conclusions: A high glycemic index diet is a risk factor for early AMD, the recognised precursor of sight-threatening late AMD. Low glycemic index foods such as oatmeal may protect against early AMD.

Introduction

Age-related macular degeneration (AMD) affects over 10% of persons aged 50 years or older and is the most frequent cause of incurable blindness in the United States and elsewhere[238;324;325]. AMD has early and late forms, with early AMD signs the precursor for sight-threatening late AMD. Dietary parameters have long been implicated as possible risk factors for AMD. The Age-Related Eye Disease Study (AREDS) demonstrated that high dose zinc and anti-oxidant supplementation reduced progression from early to late AMD.[21;326] However, few clinical trials have investigated primary prevention of early AMD and their findings have been equivocal.[66;67]

Dietary glycemic index is commonly used to characterise the postprandial blood glucose response to consumption of carbohydrates, now recognised as an important factor for cardiovascular disease.[297;327] The glycemic index ranks carbohydrate quality from 0 (low glycemic response) to 100 (high glycemic response), based on the blood glucose response 2 hours after consuming 50 grams of a carbohydrate food relative to the response from consuming 50 grams of glucose.[298] The index therefore provides a global summary measure of the rate of digestion and absorption of that carbohydrate food. Diets with a high glycemic index are associated with an increased risk of coronary heart disease, stroke and type 2 diabetes.[299;328-330]

It is unknown if high glycemic index diets are associated with risk of AMD. Two cross-sectional studies reported an association between dietary consumption of carbohydrates with higher glycemic index and AMD,[115;117] but prospective studies are lacking. In this population-based prospective cohort study, we examined the associations of dietary glycemic index and long-term risk of AMD. We specifically investigated the independent

effect of dietary fibre intake, given known inter-relationships between glycemic index and fibre,[331] and also investigated food groups that could underlie potential associations.

Methods

Study population

We conducted a population-based cohort study of vision, common eye diseases and other health outcomes in an urban, predominantly Caucasian population aged 49 years or older in Blue Mountains, west of Sydney, Australia. At baseline in 1992-4, 3654 participants (82.4% response) were examined.[211;237] Participants were examined every 5 years; 2335 (75.1% of survivors) at the second examination in 1997-9, and 1,952 (76.5% of survivors) at the third in 2002-4. The study adhered to recommendations of the Helsinki Declaration and was approved by the Sydney West Area Human Ethics Committee. Written, informed consent was obtained from all participants.

AMD definition

At each visit, 30° stereoscopic retinal photographs of the macula and other retinal fields of both eyes were taken, as described previously.[211] Details of the photographic grading for AMD lesions have been reported.[211] It closely followed the Wisconsin Age-Related Maculopathy Grading System.[186] All photographs taken at each examination had an initial masked grading. Assessments of inter- and intra-grader reliability showed good agreement.[211] Side-by-side grading of the baseline and 5-year photographs[230] and of the baseline and 10-year photographs, was then performed for participants with any AMD lesions identified at either follow-up examination.

Early AMD was defined, in the absence of late AMD, as presence at the macula of either: (1) large (>125 μm diameter) indistinct soft (or reticular) drusen or (2) both large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or hypopigmentation)[190;211] Late AMD was defined to include either neovascular AMD or geographic atrophy (GA), the two late-stage lesions described in the International AMD classification.[190] All late AMD cases detected from each examination were adjudicated and confirmed by a retinal specialist (PM).

Incident early AMD was defined by new appearance of early AMD lesions at follow-up examinations.[238] Participants with either distinct soft drusen or retinal pigmentary abnormalities at the baseline examination, but not both, who went on to develop complementary lesions that together comprised early AMD were included as incident early AMD cases.[238] Incident indistinct soft drusen or incident retinal pigmentary abnormalities were defined similarly among persons without early or late AMD. Incident late AMD was defined by the new appearance at follow-up of neovascular AMD or GA.

Dietary Assessment

A standardised interview and examination was performed and participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular from a Willett questionnaire,[213] that incorporated a nine-category frequency scale and standard portion size estimates. This FFQ had reasonable concurrent validity when validated against 4-day weighed food records collected on three occasions in one year (n=79).[214] The validation yielded an energy-adjusted Spearman coefficient of 0.82 between self-reported and weighed food records, and correctly classified 85% to within one quintile difference for dietary fibre.[214] The corresponding coefficient for GI was 0.57 and

correct classification of subjects to within one quintile difference for GI was 74%.[298] Dietary intakes were estimated using Australian Tables of Food Composition (NUTTAB 90).[216]

Glycemic index data were obtained from the Sydney University Glycemic Index Research Service (SUGiRS) online database (www.glycemicindex.com) and published values.[220] In total, 88.9% of glycemic index values were obtained from published values and 11.1% were interpolated from similar food items. An overall glycemic index value for each participant's diet was calculated by summating the weighted glycemic index of individual foods in the diet, with the weighting proportional to the contribution of individual foods to total carbohydrate intake. The consumption of breakfast cereals, collected in the FFQ, was used to enhance accuracy of the GI calculations.

We also extracted data on the fibre contribution from both breads and cereals.

Assessment of confounders

The interview included questions about past medical history, including physician-diagnosed history of stroke or myocardial infarction, and lifestyle factors like smoking. Higher educational achievement was defined as attainment of qualifications (certificate, diploma or degree) after leaving school. A single measure of systolic and diastolic blood pressure using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Mean arterial blood pressure was defined as 0.33×systolic blood pressure +0.67×diastolic blood pressure. Body mass index (BMI) was calculated as weight(kg) /height $(m)^2$. Diabetes was defined either from past history of diabetes and current diabetes treatment, or from fasting plasma glucose levels ≥7.0mmol/L at examination, using the World Health Organization diabetes classification.[224] Fasting blood samples were processed on the same day for white

cell count, total cholesterol and high-density lipoprotein (HDL)-cholesterol by the Institute of Clinical Pathology, Westmead Hospital.

Study Sample

The baseline cohort consisted of 3654 predominantly Caucasian participants who were aged 49 years or older, with 43.3% being male. At the 10-year follow-up examinations there were 1952 participants, 1103 (30.2% of original cohort) of participants had died, 375 (10.3%) moved from the study area, and 224 (6.1%) refused to participate. Retinal photographs were obtained for both eyes in 98%, or for at least one eye in 99%, of the baseline and 5-year participants,[230] and for both eyes in 85% (1649/1952), or for at least one eye in 87% (1689/1952), of the 10-year participants. Those lost to follow-up tended to be younger, to have lower socioeconomic status and to smoke, but were less likely to have coronary heart disease.[230]

The FFQ was attempted and returned by 3267 baseline participants (89.4%), with 2897 (79.3% of total participants) having sufficiently complete and plausible FFQ data for analysis. Subjects were excluded if over 12 questions were missing, if an entire page remained blank, or if daily energy intakes were <2500 kJ or >18,000 kJ.[187;214] Participants without usable FFQ data were more likely to be older (mean age 69.3 years vs. 65.3 years), or current smokers (17.7% vs. 14.2%) than those with usable FFQs.

We conducted analyses initially on the whole cohort and then excluded persons with diabetes, on the basis that persons with diabetes are likely to be misclassified in GI due to their unpredictable glycemic responses. The baseline study sample thus consisted of 2641 participants (72.3%) who had reliable dietary assessment, gradable fundus photographs, participated in at least 1 follow-up examination and did not have diabetes.

Statistical Methods

Statistical analyses were performed using Statistical Analysis Software (version 9, SAS Institute, Cary, NC). We examined the association between baseline mean dietary glycemic index, consumption of carbohydrates and fibre as well as specific foods and the 10-year incidence of both early and late AMD. Glycemic index, carbohydrates and fibre variables were adjusted for total energy intake using the Willett residual method.[241] Subject intakes were divided into quartiles for glycemic index, macronutrients and food groups.

Person-specific incidence rates were calculated using Kaplan-Meier product-limit survival estimates, to incorporate information from the 5- and 10-year examinations. Cumulative incidence was estimated as (1-Kaplan-Meier estimate) and expressed as a percentage. Discrete linear logistic models were used to assess relationships between dietary variables and incident early or late AMD at either of the 2 follow-up time points. The following potential confounders were considered: age, gender, mean arterial blood pressure, BMI, smoking, HDL-cholesterol, post secondary school qualifications, past history of coronary heart disease or stroke, and consumption of fish, total vegetables, fruit and total fat. Micronutrient variables, vitamins C and E, beta-carotene, zinc, lutein, zeaxanthin and folate replaced total vegetables, fruit and total fat in alternative models. Relative risks (RR) and 95% confidence intervals (CI) are presented.

Results

Over the 10-year period, incident early AMD developed in 208 of 1810 persons at risk (cumulative incidence, 14.1%) and late AMD developed in 54 of 1913 persons at risk (cumulative incidence, 3.7%). The mean (SD) energy-adjusted glycemic index of foods consumed in this population was 56.6 (4.5) in persons without diabetes. **Table 4.1** shows

that participants with incident (early and late) AMD were older and more likely to be male at baseline than those without AMD.

Table 4.2 shows the characteristics of the population by glycemic index quartiles. Male gender, qualification level, smoking status, HDL-cholesterol level, white cell count, and consumption of vegetables, fish and macro- and micronutrients, differed across these quartiles. Correlations between dietary variables were moderate to low -0.2 to 0.4.

Table 4.3 shows the associations between mean dietary glycemic index, cereal fibre, consumption of breads and cereals and the 10-year incidence of early AMD, in persons without diabetes. Subjects with the highest compared to the lowest quartile of mean dietary glycemic index at baseline had a 77% higher 10-year risk of early AMD (p_{trend}=0.02). This increased risk was unchanged by including cereal fibre in the model. On the other hand, subjects consuming the highest compared to the lowest quartile of cereal fibre had a 68% reduction in their 10-year risk of early AMD (p_{trend}=0.05). A similar reduction in risk of early AMD was evident for increasing consumption of breads and cereals (p_{trend}=0.03).

Analysis of the entire cohort (inclusion of persons with diabetes) produced similar, but borderline significant findings. Furthermore, inclusion of white cell count in the statistical models attenuated the observed effect sizes, likely due to the reduced number as there were some 20% of participants who did not have this blood test performed. Stratification by age showed that in persons younger than age 70 years (at the baseline examination), the relationship between early AMD and glycemic index (quartile 4 vs. quartile 1, 78% increased risk of early AMD) or cereal fibre (quartile 4 vs. quartile 1, 54% reduced risk of early AMD) was strengthened and remained significant. In persons over age 70 years, the results were markedly attenuated, with no trends demonstrated, and became non-significant.

Table 4.4 shows multivariate-adjusted associations between mean dietary glycemic index, cereal fibre, consumption of breads and cereals and the 10-year incidence of indistinct soft drusen and pigmentary abnormalities, the two cardinal signs of early AMD. The highest compared to the lowest quartile of mean dietary glycemic index at the baseline examination predicted a 68% higher 10-year risk of indistinct soft drusen (p_{trend}=0.04). The highest compared to lowest quartile of cereal fibre (p_{trend}=0.01) and breads and cereal consumption (p_{trend}=0.04) predicted a 39% and 47% reduction, respectively in the 10-year risk of indistinct soft drusen. Relatively similar reduction (by 39% or 31%) in the 10-year risk of retinal pigmentary abnormalities, was predicted by the highest quartile of cereal fibre (p_{trend}=0.04) and breads and cereal consumption (p_{trend}=0.04), respectively.

We further examined dietary composition in the breads and cereals group. The highest mean intakes within this group were mostly of relatively low glycemic index foods such as oatmeal and wholemeal/mixed grain bread. For example, the daily mean consumption of oatmeal (mean 60.3 grams/day) was substantially greater than the mean consumption of other breakfast cereals (mean 32.9 grams/day). Similarly, the mean consumption of wholemeal/mixed grain bread (mean 48.0 grams/day) was greater than for white bread, a relatively high glycemic index food (mean 23.1 grams/day).

Overall carbohydrate consumption was not associated with the incidence of early AMD or its component lesions. No significant relationships were found between total dietary fibre or its separate vegetable fibre or fruit fibre components, nor between individual vegetable or fruit consumption and the incidence of early AMD or its component lesions.

No significant associations were found between the mean dietary glycemic index of foods consumed, cereal fibre, carbohydrates and the 10-year incidence of late AMD (data not shown).

Discussion

Diet is one of few modifiable risk factors for AMD, the major cause of blindness among elderly persons in the US. In this prospective population-based study, we demonstrate that diets with higher glycemic index were associated with increased 10-year risk of early AMD and its key component lesion, indistinct soft drusen. Conversely, greater consumption of cereal fibre was associated with a reduced risk of early AMD and its components. We identified specific food groups: breads and cereals consumption, which might underlie these relationships. These associations were independent of smoking and traditional AMD risk factors.

The calculated glycemic index of carbohydrates is commonly used to determine its "dietary value", since carbohydrates are critical macronutrients influencing insulin secretion and postprandial glycemia, now known to be important factors in pathogenesis of diabetes and cardiovascular disease. Consistent with this hypothesis, we found no association between total consumption of carbohydrates and risk of early AMD, suggesting that it is not the quantity of carbohydrates per se, but possibly their post-prandial effects, that are important.

Our results are based on the exclusion of persons with diabetes. Analysis of the entire cohort attenuated the significance level of findings. It is likely that the inclusion of persons with diabetes led to some misclassification in GI, tending to bias our results towards the null. Persons with diabetes are likely to have unpredictable glycemic responses, making it

difficult to classify these subjects based on the glycemic values extrapolated from persons without diabetes.

To the best of our knowledge, there are only two studies, both cross-sectional, which have examined this relationship. The Nurses Health Study (NHS) study found that mean dietary glycemic index was related to pigmentary abnormalities but not to drusen.[115] However, we found no significant prospective association between glycemic index and long-term risk of pigmentary abnormalities. The reasons for the difference in findings with our study are unclear, although differing study methods, particularly AMD definitions, may be relevant. Analysis of AREDS data showed a relationship between large drusen and highest quintile of glycemic index as well as a positive relation between mean glycemic index and increasing severity of disease.[117]

Our findings have a sound biological basis. Early AMD signs such as soft drusen may result from oxidative damage in the light- and oxygen-rich milieu of the retina,[31] or by inflammation and activation of the complement cascade.[30;332] It is possible that either or both of these two pathogenic mechanisms may be activated by higher glycemic index diets. Normal levels of glycemia tend to depress plasma antioxidant capacity[304] and hyperglycaemia has been shown to generate oxidative stress.[305;306;317;333] In diabetes, the oxidative stress generated by hyperglycemia has been shown to activate all pathways leading to diabetes complications, including the polyol and hexosamine pathways, the formation of advanced glycation end products and the activation of protein kinase C.[317] In the case of AMD, it seems likely that oxidative stress results in protein modifications that contribute to the development of drusen[79;334] In relation to inflammation, a recent study showed that a high glycemic load diet predicted higher levels of C-reactive protein, an inflammatory mediator also found in drusen.[30;182]

Evidence for a role for advanced glycosylation end products (AGEs) in AMD pathobiology is also accumulating. AGEs are important pathological by-products of hyperglycemia and have been found to accumulate in the outer retina with increasing age.[116] Higher AGE levels are found in persons with AMD, and are also a component of drusen.[116] It is thought that the vascular endothelium is exquisitely sensitive to hyperglycaemia because of its inability to control glucose transport across the membrane.[317] AGEs accumulate in the endothelium contributing to both endothelial dysfunction and permeability, a mechanism proposed for the increased risk of stroke in persons consuming high glycemic index diets.[302] Recent studies have shown links between stroke, cardiovascular disease and AMD - one of the possible mechanisms may be hyperglycaemia-induced damage to the vascular endothelium.[335;336]

We showed that cereal fibre consumption reduced the long-term risk of early AMD. To our knowledge, this association has not been investigated previously. Cereal fibre can also reduce the glycemic response to subsequent meals by a second meal effect.[337] Lower levels of post-prandial glycemia may thus represent a common mechanism for the beneficial effects observed from both glycemic index and cereal fibre.

Strengths of our study include its prospective nature with long-term follow-up of a stable population-based sample, with reasonable follow-up, the use of high quality stereoscopic retinal photography with validated grading to assess macular conditions, including side-by-side comparisons of the baseline and follow-up examination photographs and the reliable categorisation of the glycemic index of a wide range of Australian foods, contrasting with other studies in which this index is largely extrapolated.[220]

Our study has several limitations. We had insufficient power to demonstrate relationships between mean glycemic index and the incidence of late AMD. A relatively high proportion

of participants with missing FFQ data were likely to be older and current smokers. This may explain the lack of baseline differences between participants with and without incident AMD, as the missing data might have diluted the associations observed since smokers and older persons were more likely to have AMD. Finally, the use of a single blood pressure measurement may not accurately reflect a participant's 'usual' blood pressure with resultant non-differential misclassification of some participants.

As healthy behaviours such as non-smoking and greater fruit and vegetable consumption were associated with lower mean glycemic index diets in our study (Table 4.1), the overall glycemic index of foods consumed by individuals may be a marker for healthy dietary and lifestyle patterns, rather than representing a causal pathway. We consume a combination of various foods simultaneously, but not in isolation. However our analyses address the associations of AMD with food components in relative isolation, with adjustment for energy and only a limited number of other food elements and lifestyle factors. Additionally, people may potentially change their diets over time, and our dietary data, collected at one point in time, likely suffer from a range of measurement errors. It remains a challenge to nutritional epidemiologists to summarise and group patterns of dietary intake and also cover all aspects of diet. Currently there are no widely accepted approaches in this regard. We have attempted to address some of this by controlling for many dietary and lifestyle factors in the analysis. We also excluded persons who were likely to have modified their diets, such as persons with diabetes, who may also have unpredictable glycemic responses to similar foods compared to the general population. We feel that our study findings will need to be validated by future studies using better approaches to eliminate potential residual confounding effects. Support for our study findings at this stage, however, arises from the consistency of the findings across the three studies, ours and the two others[115;117], that include both cross-sectional and longitudinal observations.

Finally, in our population higher glycemic index was found associated with an increase in stroke-related mortality (though not all-cause or coronary heart disease mortality). Although survival bias could have diluted our results, this effect is likely to be relatively modest, as the number of stroke deaths was small (n=95).

Early AMD is the major predictor of progression to late AMD and there are few current effective preventive or therapeutic strategies that target these early signs.[31;338;339] Primary prevention of early AMD lesions will substantially reduce the number of people who develop sight-threatening late AMD. While antioxidant supplements may delay progression from early to late AMD,[21] findings from other randomised controlled trials on primary prevention of early AMD have been equivocal.[66;67] Our study has therefore potential implications for prevention of early AMD in the population.

In summary, we show that a diet with a high mean glycemic index is a risk factor for early AMD. Conversely cereal fibre may reduce the risk of early AMD. We demonstrate specific foods that may lower this risk. Our findings require replication in prospective studies in other populations. AMD is currently responsible for around 14 million cases of blindness or severe visual impairment worldwide.[340] Given its significant public health impact, recommendations for lower glycemic index diets could assist in preventing AMD on a population-wide basis.

Table 4.1. Baseline characteristics of the study sample (n=2641)*

Characteristics	Incident age-related macular degeneration† (n=262)	No incident age-related macular degeneration† (n=2379)	p-value
Age (yrs)	68.7 (7.7)	63.1 (8.2)	<0.0001
Male gender (%)	44.3	37.0	0.04
Mean arterial blood pressure (mmHg)	104.8 (11.9)	103.6 (11.9)	0.13
Body-mass index	26.0 (4.1)	26.4 (4.5)	0.22
Current smoker (%)	12.7	10.5	0.36
History of coronary heart disease (%)	13.2	17.4	0.09
History of stroke (%)	2.5	5.0	0.03
Fasting serum cholesterol (mmol/L)	6.1 (1.0)	6.0 (1.1)	0.72
Consumption of fish (grams/day)	25.3 (24.5)	27.7 (27.4)	0.23
Zinc (mg/day)	12.0 (0.2)	11.8 (0.1)	0.24
Vitamin C (mg/day)	337.1 (25.9)	346.1 (9.3)	0.74
Vitamin E (mg/day)	50.2 (7.5)	38.4 (2.7)	0.14
Betacarotene (µg/day)	7641.5 (303.9)	7409.5(109.2)	0.47
Lutein & zeaxanthin (µg/day)	827.7(11.5)	833.8 (32.1)	0.86

*Data are expressed as means (SD) unless otherwise indicated; chi-square and t-test used to assess differences between persons with incident age-related macular degeneration and persons without incident age-related macular degeneration.

†Incident age-related macular degeneration includes both early and late forms

Table 4.2. Factors associated with age-related macular degeneration at the baseline Blue Mountains Eye Study examination by mean dietary glycemic index quartiles (n=2641).*

Characteristics	Glycemic Index quartile 1 (51.8±2.5) (n=646)	Glycemic Index quartile 2 (55.2±0.8) (n=664)	Glycemic Index quartile 3 (57.7±0.8) (n=662)	Glycemic Index quartile 4 (61.4±2.8) (n=669)	p-value†
Age (yrs)	64.0 (9.3)	65.5 (9.2)	65.0 (9.1)	65.0 (9.6)	0.11
Male gender (%)	29.8	39.4	47.1	59.4	<0.001
Post secondary school qualifications (%)‡	55.9	52.9	47.6	43.2	<0.001
Mean arterial blood pressure (mmHg)	102 (12.2)	103 (12.3)	104 (12.3)	103 (12.1)	0.12
Body-mass index	25.7 (4.5)	25.7 (4.3)	25.7 (4.8)	25.4 (4.6)	0.57
Current smoker (%)	9.34	11	14.2	22.4	<0.001
History of coronary heart disease (%)	14.7	16	13.4	15.7	0.94
History of stroke (%)	3.56	4.8	5.49	4.12	0.5
Fasting serum cholesterol (mmol/L)	6.10 (1.1)	6.00 (1.0)	6.00 (1.0)	5.90 (1.1)	0.14
Fasting HDL-cholesterol (mmol/L)	1.40 (0.4)	1.40 (0.4)	1.30 (0.4)	1.30 (0.5)	0.02
White cell count ($x10^9$)	6.10 (1.7)	6.25 (1.7)	6.20 (1.7)	6.40 (1.8)	<0.001

*Data are expressed as means (SD) unless otherwise indicated; glycemic index energy-adjusted; chi-square and ANCOVA used to assess trends across quartiles.

†p for trend across categories <0.05

‡Any post school qualification (inc. trade certificate, diploma, degree)

§Antioxidant amounts include both diet (energy-adjusted) and supplements; total fat and fibre energy-adjusted.

Table 4.2. continued.

Characteristics	Glycemic Index quartile 1 (51.8±2.5) (n=646)	Glycemic Index quartile 2 (55.2±0.8) (n=664)	Glycemic Index quartile 3 (57.7±0.8) (n=662)	Glycemic Index quartile 4 (61.4±2.8) (n=669)	p-value†
Consumption of:					
vegetables (grams/day)	434 (198)	428 (191)	410 (180)	363 (214)	0.03
fish (grams/day)	30.9 (30.2)	30.7 (31.0)	25.7 (26.2)	22.4 (21.6)	<0.001
Dietary intake of:§					
total fat (grams/day)	74.8 (15.1)	77.6 (14.2)	77.1 (13.5)	75.9 (13.0)	0.27
total fibre (grams/day)	31.7 (9.4)	28.8 (8.2)	25.9 (7.1)	22.4 (6.8)	<0.001
vitamin E (mg/day)	9.60 (141)	8.53 (122)	7.69 (93)	7.14 (88)	<0.001
vitamin C (mg/day)	256 (477)	222/(355)	191 (354)	156 (296)	<0.001
beta-carotene (µg/day)	7435 (4982)	7161 (4282)	6462 (4167)	5799 (4340)	<0.001
zinc (mg/day)	12.3 (2.3)	11.9 (2.1)	11.4 (2.2)	11.0 (2.3)	<0.001
lutein & zeaxanthin (µg/day)	883 (537)	778 (471)	713 (485)	587 (414)	<0.001
folate (µg/day)	392 (315)	357 (180)	337 (366)	317 (197)	<0.001

*Data are expressed as means (SD) unless otherwise indicated; glycemic index energy-adjusted; chi-square and ANCOVA used to assess trends across quartiles.

†p for trend across categories <0.05

‡Any post school qualification (inc. trade certificate, diploma, degree)

§Antioxidant amounts include both diet (energy-adjusted) and supplements; total fat and fibre energy-adjusted.

Table 4.3. Multivariate adjusted associations between mean dietary glycemic index, cereal fibre, breads and cereals and the 10 year incidence of early age-related macular degeneration (n=2641).*

	Early age-related macular degeneration					
Variable	No. at risk	Cases	CI†	Relative Risk (95%CI)‡	Relative Risk (95%CI)§	Relative Risk (95%CI)‖
Mean dietary glycemic index (median)						
Q1 (51.9)	473	43	10.3	1	1	1
Q2 (55.2)	459	55	13.9	1.30 (0.85-1.97)	1.39 (0.91-2.14)	1.40 (0.91-2.14)
Q3 (57.7)	455	52	13.4	1.39 (0.93-2.09)	1.43 (0.92-2.22)	1.40 (0.90-2.18)
Q4 (61.3)	423	58	17.4	1.70 (1.15-2.64)	1.77 (1.13-2.78)	1.67 (1.06-2.64)
p trend				0.02	0.02	0.04

*Glycemic index and cereal fibre energy-adjusted; excludes persons with diabetes; discrete linear logistic regression used to assess relative risk of 10 year incident early AMD.

†CI=cumulative incidence

‡Adjusted for age and gender

§Additional adjustment for mean arterial blood pressure, body-mass index, smoking, HDL-cholesterol, qualification level, past history of myocardial infarction or stroke, fish consumption and total vegetable, fruit and total fat (energy-adjusted) intakes. Micronutrient variables, vitamins C and E, beta-carotene, zinc, lutein, zeaxanthin and folate replaced total vegetables and total fat in alternative models, results were similar.

‖Additional adjustment for cereal fibre

#Highest mean intakes within the breads and cereals group were mostly of relatively low glycemic index foods such as oatmeal.

Table 4.3. continued.*

Variable	Early age-related macular degeneration					
	No. at risk	Cases	CI†	Relative Risk (95%CI)‡	Relative Risk (95%CI)§	Relative Risk (95%CI)‖
Cereal fibre (median, grams)						
Q1 (2.8)	412	51	15.4	1	1	N/A
Q2 (5.3)	448	55	15.0	0.87 (0.59-1.30)	0.88 (0.59-1.33)	
Q3 (7.8)	472	53	13.0	0.73 (0.49-1.10)	0.75 (0.49-1.14)	
Q4 (12.3)	478	49	11.6	0.71 (0.47-1.06)	0.68 (0.44-1.04)	
p trend				0.07	0.05	
Breads and cereals (median, grams)#						
Q1 (82.6)	413	55	16.1	1	1	N/A
Q2 (150.5)	446	57	15.0	0.92 (0.62-1.36)	0.94 (0.63-1.41)	
Q3 (231.8)	491	46	11.1	0.72 (0.48-1.08)	0.75 (0.49-1.14)	
Q4 (376.0)	460	50	12.7	0.68 (0.46-1.01)	0.67 (0.44-1.02)	
p trend				0.03	0.03	

*Glycemic index and cereal fibre energy-adjusted; excludes persons with diabetes; discrete linear logistic regression used to assess relative risk of 10 year incident early AMD.

†CI=cumulative incidence

‡Adjusted for age and gender

§Additional adjustment for mean arterial blood pressure, body-mass index, smoking, HDL-cholesterol, qualification level, past history of myocardial infarction or stroke, fish consumption and total vegetable, fruit and total fat (energy-adjusted) intakes. Micronutrient variables, vitamins C and E, beta-carotene, zinc, lutein, zeaxanthin and folate replaced total vegetables and total fat in alternative models, results were similar.

‖Additional adjustment for cereal fibre

#Highest mean intakes within the breads and cereals group were mostly of relatively low glycemic index foods such as oatmeal.

Table 4.4. Multivariate adjusted associations between mean dietary glycemic index, cereal fibre, breads and cereals and the 10 year incidence of the two hallmark lesions of early age-related macular degeneration (n=2641).*

	Indistinct soft drusen†					Pigmentary abnormalities				
Variable	No. at risk	Cases	Cumulative Incidence	Relative Risk (95%CI)‡	Relative Risk (95%CI)§	No. at risk	Cases	Cumulative Incidence	Relative Risk (95%CI)‡	Relative Risk (95%CI)§
Mean dietary glycemic index (median)										
Q1 (51.9)	477	37	9.0	1	1	447	84	22.2	1	1
Q2 (55.2)	462	45	11.3	1.38 (0.89-2.13)	1.33 (0.83-2.12)	431	83	22.8	0.99 (0.72-1.35)	1.01 (0.72-1.41)
Q3 (57.7)	459	44	10.9	1.31 (0.83-2.08)	1.41 (0.87-2.27)	435	80	22.6	0.95 (0.68-131)	0.98 (0.69-1.38)
Q4 (61.3)	423	47	14.1	1.67 (1.07-2.63)	1.68 (1.03-2.74)	393	77	23.7	1.05 (0.75-1.46)	1.08 (0.75-1.55)
p trend				0.04	0.04				0.89	0.75
Cereal fibre (median, grams)										
Q1 (2.8)	415	48	14.4	1	1	388	92	29.3	1	1
Q2 (5.3)	452	46	12.3	0.72 (0.47-1.10)	0.75 (0.48-1.16)	420	60	17.1	0.51 (0.36-0.72)	0.49 (0.34-0.70)
Q3 (7.8)	472	36	8.8	0.51 (0.32-0.79)	0.51 (0.32-0.82)	452	88	22.7	0.66 (0.48-0.90)	0.69 (0.50-0.96)
Q4 (12.3)	482	43	10.1	0.64 (0.42-0.97)	0.61 (0.39-0.96)	446	84	22.8	0.62 (0.45-0.85)	0.61 (0.43-0.85)
p trend				0.02	0.01				0.02	0.04

*Glycemic index and cereal fibre energy-adjusted; excludes persons with diabetes, discrete linear logistic regression used to assess relative risk of the 10 year incidence of the component lesions of early AMD.

†Also includes the relatively fewer cases of reticular drusen

‡Adjusted for age and gender

§Additional adjustment for mean arterial blood pressure, body-mass index, smoking, HDL-cholesterol, qualification level, past history of myocardial infarction or stroke, fish consumption and total vegetable, fruit and total fat (energy-adjusted) intakes. Micronutrient variables, vitamins C and E, beta-carotene, zinc, lutein, zeaxanthin and folate replaced total vegetables and total fat in alternative models, results were similar.

‖Highest mean intakes within the breads and cereals group were mostly of relatively low glycemic index foods such as oatmeal.

Table 4.4. continued*

Variable	Indistinct soft drusen†					Pigmentary abnormalities				
	No. at risk	Cases	Cumulative Incidence	Relative Risk (95%CI)‡	Relative Risk (95%CI)§	No. at risk	Cases	Cumulative Incidence	Relative Risk (95%CI)‡	Relative Risk (95%CI)§
Breads and cereals (median, grams)‖										
Q1 (82.6)	416	52	14.9	1	1	387	86	27.1	1	1
Q2 (150.5)	451	47	12.1	0.81 (0.54-1.23)	0.81 (0.53-1.24)	422	80	22.7	0.74 (0.54-1.03)	0.75 (0.53-1.05)
Q3 (231.8)	492	35	8.4	0.58 (0.37-0.91)	0.60 (0.38-0.95)	465	78	20.5	0.72 (0.52-0.99)	0.71 (0.50-0.99)
Q4 (376.0)	462	39	10	0.57 (0.37-0.88)	0.53 (0.33-0.83)	432	80	21.5	0.69 (0.50-0.95)	0.69 (0.49-0.97)
p trend				0.08	0.04				0.04	0.04

*Glycemic index and cereal fibre energy-adjusted; excludes persons with diabetes, discrete linear logistic regression used to assess relative risk of the 10 year incidence of the component lesions of early AMD.

†Also includes the relatively fewer cases of reticular drusen

‡Adjusted for age and gender

§Additional adjustment for mean arterial blood pressure, body-mass index, smoking, HDL-cholesterol, qualification level, past history of myocardial infarction or stroke, fish consumption and total vegetable, fruit and total fat (energy-adjusted) intakes. Micronutrient variables, vitamins C and E, beta-carotene, zinc, lutein, zeaxanthin and folate replaced total vegetables and total fat in alternative models, results were similar.

‖Highest mean intakes within the breads and cereals group were mostly of relatively low glycemic index foods such as oatmeal.

Chapter 9 - Eggs and the risk of age-related macular degeneration

Abstract

Context: Studies have shown that dietary modification can potentially influence the genetic risk for age-related macular degeneration (AMD), the leading cause of visual loss among people aged 65+ years in the United States. Eggs, rich in lutein and zeaxanthin, may alter this risk. Currently evidence in this regard from prospective or interventional studies is lacking.

Objective: To examine the association between egg consumption and the 10-year incidence of AMD.

Design, setting, participants: Population-based, prospective cohort study of persons aged 49+ years living west of Sydney, Australia. Of 3,654 participants at baseline (1992-4), 2,335 (75.1% of survivors) were re-examined after 5 years and 1,952 (75.6% of survivors) after 10 years. AMD was graded from stereoscopic retinal photographs using the Wisconsin Grading System. Incident cases were confirmed using side-by-side grading methods. Baseline dietary information was collected using a validated food frequency questionnaire, adapted from a Willet questionnaire. Consumption of eggs was recorded in grams and divided into quartiles.

Main outcome measures: 10-year incident early and late AMD

Results: Over 10 years, 208 of 1810 persons at risk (cumulative incidence 13.6%) developed early AMD, and 54 of 1913 persons at risk developed late AMD (cumulative incidence 3.6%). After adjusting for age, smoking, other risk factors and dietary constituents, increasing quartile of egg consumption was associated with a reduced risk of late AMD (relative risk, RR 0.29, 95% confidence interval CI, 0.12–0.70, comparing 4^{th} to 1^{st} quartile, p_{trend}=0.01). After stratification by late AMD type, this protective association was significant for geographic atrophy (RR 0.21, 95% CI, 0.05-0.79, p_{trend}=0.03), but marginally non-significant for neovascular AMD (RR 0.34, 95% CI, 0.12-1.09, p_{trend}=0.07).

Conclusions: We found that high consumption of eggs was associated with a 70% reduction in the long-term risk of late macular degeneration. This protective effect of eggs on geographic atrophy has an important clinical implication, given the lack of treatments available for this condition.

Introduction

Whilst clinical trials have documented that lutein and zeaxanthin supplements benefited patients with age-related macular degeneration (AMD),[68;341-344] the effect of consumption of egg yolk, the best dietary source of these carotenoids, has not been studied.[71;341;345]

Lutein and zeaxanthin are xanthophyll pigments present in the macula, and considered important elements with antioxidant functions in addition to the filtration of ultraviolet light.[70] Loss of these pigments was found to be correlated with atrophy of the macula, while supplements of these antioxidants have been shown in a number of studies to improve macula pigment density and possibly visual function.[68;71;343;344;346]

A large randomized controlled trial, the Age-Related Eye Disease Study (AREDS), is currently examining the effect of oral tablet supplementation of lutein and zeaxanthin, in addition to the original AREDS formulation, on delaying the progression to advanced stage AMD.[83] However, the study results will not be available for some time and there is evidence suggesting that lutein-enriched egg yolk has a higher lutein bioavailability than oral supplements of lutein and zeaxanthin.[347] Eggs have also been shown to improve serum lutein and zeaxanthin to a greater level than other *food* sources of these pigments.[345;347]

In this report, we aimed to examine egg consumption and the 10-year risk of AMD in a population-based cohort of older persons.

Methods

The Blue Mountains Study is a population-based cohort study of an Australian population aged 49 years or older.[211] At baseline in 1992-4, 3654 participants (82.4% response) were

examined, 2335 (75.1% of survivors) were re-examined at 5 years (1997-9) and 1,952 (76.5% of survivors) at 10 years (2002-4). Institutional ethics committee approval and written consent was obtained.

Details of the photographic grading from retinal photographs for AMD lesions have been reported.[211;230] It closely followed the Wisconsin Age-Related Maculopathy Grading System.[186;238] Incident late AMD was defined if neovascular AMD or geographic atrophy was detected at either follow-up examination in persons free of these lesions at baseline. Incident early AMD was defined if soft indistinct or reticular drusen, or combined soft distinct drusen and retinal pigment abnormality, were present in persons free of early and late AMD at baseline.[230;237]

A standardised interview and examination was performed and participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular, from a Willett questionnaire,[213] that incorporated a nine-category frequency scale. Levels of consumption of eggs were estimated using the food frequency questionnaire, converted to grams and then divided into quartiles.

Discrete linear logistic models were used to assess relationships between egg consumption and 10-year incident early or late AMD at either of the 2 follow-up time points. Dietary variables were adjusted for total energy intake using the Willett residual method.[241] Covariates were: age, gender, mean arterial blood pressure, BMI, smoking, HDL-cholesterol, white cell count, post secondary school qualifications, presence of diabetes, past history of coronary heart disease or stroke, and consumption of fish and antioxidants.

Results

Over the 10-year period, incident early AMD developed in 208 of 1810 persons at risk (cumulative incidence, 13.6%) and late AMD developed in 54 of 1913 persons at risk (cumulative incidence 3.6%), after excluding those with missing FFQ data or ungradable retinal photographs. **Table 4.5** shows the characteristics of the population by egg consumption quartiles. Age, male gender, history of cardiovascular disease, white cell count, consumption of fish, vegetables and beta-carotene differed among the quartile groups.

Table 4.6 shows multivariate-adjusted associations between egg consumption and the 10-year incidence of late AMD. Subjects with the highest egg consumption (corresponding to 6 eggs per week) compared to the lowest quartile (corresponding to 0 eggs per week) at baseline had a 74% reduction in 10-year risk of late AMD (p_{trend}=0.01). There was no relationship demonstrated with early AMD lesions (data not shown).

Table 4.7 shows multivariate-adjusted associations between egg consumption and the 10-year incidence of the two cardinal signs of late macular degeneration lesions, geographic atrophy and neovascular degeneration. Participants in the highest quartile of egg consumption were significantly protected from the development of geographic atrophy (relative risk, RR, comparing highest vs. lowest quartile, 0.21, 95% confidence interval, CI, 0.05-0.79, p_{trend}=0.03). There was the suggestion of a lower risk of development neovascular degeneration, but the trend was non-significant (comparing highest vs. lowest quartile, RR, 0.34, 95% CI, 0.12-1.09, p_{trend}=0.07).

Discussion

In this prospective population-based study, we demonstrate that egg consumption is protective against the incidence of late AMD and its key component lesion, geographic atrophy. There are currently no treatments for atrophic AMD. This association was independent of traditional AMD risk factors including cigarette smoking, cardiovascular parameters, inflammatory variables, socioeconomic status and other dietary constituents. We did not find a relationship between egg consumption and early AMD.

Several trials and studies have examined the relationship between lutein and zeaxanthin and the incidence of AMD. Lutein and zeaxanthin are key components of egg yolk that may account for their protective effect against AMD. Studies have also assessed the relationship between serum levels of these pigments and incident AMD. Findings from these previous studies have been inconsistent, with some reporting benefit [73;74;191;344;348-352] and others no benefit.[76;79;80;353-358] Only one study has investigated egg consumption specifically, and found no association with early AMD.[353] There were insufficient cases of late AMD in this previous study.[353] In general, there appears to be a stronger inverse association of lutein and zeaxanthin with late, rather than early AMD in the positive studies, similar to our findings.[71;80]

Results of the large AREDS interventional trial may provide clarification with regards to the efficacy of lutein and zeaxanthin in the prevention of AMD. In the interim, clinical trials have shown that both supplements and food sources (excluding egg yolk) of lutein and zeaxanthin can increase serum levels of lutein and zeaxanthin and macula pigment density (MPOD) in healthy subjects, and MPOD and visual function in patients with atrophic AMD.[68;342-344;346;359-361] Small trials with egg supplementation have shown that it increases

serum lutein and zeaxanthin levels,[345;347;362-366] and one recent trial showed that it improved MPOD.[367]

Concerns regarding high cholesterol levels have created negative public perception of egg consumption. However, a recent study failed to show a relationship between egg consumption and coronary heart disease, and a recent review suggested that plasma cholesterol levels were not affected by restriction of consumption of eggs.[368-370]. Of late, there is increasing acknowledgement and research about the multiple beneficial effects of eggs and attempts to improve the negative public perception of eggs.[341;370-372]

Strengths of our study include its prospective nature with long-term assessment of a stable population-based sample, with reasonable follow-up (around 75% of survivors examined) and the use of high quality stereoscopic retinal photography with validated grading to assess macular conditions, including side-by-side comparisons of the baseline and follow-up examination photographs. Limitations of our study include the relatively high proportion of participants with missing FFQ data who were more likely to be older and current smokers, which might have affected the associations observed since smokers and older persons were more likely to have AMD. However persons who were older were more likely to have higher consumption of eggs, thus any effect may have been to dilute our findings, rather than change the direction of findings. As healthy behaviours such as greater fish and vegetable consumption were associated with a higher egg consumption level in our study (Table 4.5), overall egg consumption may be a marker for healthy dietary and lifestyle patterns. However, given the relatively negative publicity towards eggs for most of the last two decades, this is less likely to be an explanation for our findings. Nevertheless, we attempted to address these concerns by controlling for lifestyle and dietary factors in the analysis. Another limitation of our study is the use of a single blood pressure measurement, which

may not accurately reflect a participant's 'usual' blood pressure over time. Finally, the number of incident late AMD cases was relatively small in the highest quartile group, and therefore confirmation of our study findings in future studies is needed.

In summary, we show that high egg consumption was protective against late AMD, in particular geographic atrophy. Given observational data suggesting that lutein and zeaxanthin may be beneficial to late AMD and the bioavailability of these nutrients in eggs, our study finding has significant therapeutic implications that should be confirmed in clinical trials. The prevalence of AMD is projected to increase with the aging of the population, thus dietary modifications could assist in preventing AMD in the population.

Table 4.5. Factors associated with age-related macular degeneration at the baseline Blue Mountains Eye Study examination by quartile of egg consumption*

Characteristics	Egg Consumption quartile 1 (0 eggs/week)	Egg Consumption quartile 2 (1 egg/week)	Egg Consumption quartile 3 (3 eggs/week)	Egg Consumption quartile 4 (6 eggs/week)	p-value
Age (yrs)	65.0	64.0	66.0	65.0	0.01
Male gender (%)	37.0	40.0	43.2	52.1	<0.001
Mean arterial blood pressure (mmHg)	103.2	102.5	103.2	103.1	0.20
Current smoker (%)	11.5	12.5	10.9	15.5	0.12
History of cardiovascular disease (%)	16.5	14.4	15.5	9.3	0.003
Fasting serum cholesterol (mmol/L)	5.90	6.00	6.00	5.90	0.22
Fasting HDL-cholesterol (mmol/L)	1.40	1.40	1.40	1.40	0.60
White cell count ($x10^9$)	6.10	6.20	6.30	6.30	0.001
Consumption of:					
vegetable (grams/day)	387	410	422	440	<0.001
fish (%)†	50.9	55.8	65.3	66.3	<0.001

*Egg consumption has been converted to grams and divided into quartiles (brackets present equivalent mean number of eggs consumed by participants in these quartiles)

†Proportion of persons consuming at least one serving of fish per week

‡Antioxidant amounts include both diet (energy-adjusted) and supplements

Table 4.5. continued.*

Characteristics	Egg Consumption quartile 1 (0 eggs/week)	Egg Consumption quartile 2 (1 egg/week)	Egg Consumption quartile 3 (3 eggs/week)	Egg Consumption quartile 4 (6 eggs/week)	p-value
Dietary intake of:‡					
vitamin E (mg/day)	8.29	8.25	8	7.71	0.28
vitamin C (mg/day)	204	207	197	199	0.05
beta-carotene (µg/day)	7158	6644	6685	6372	<0.001
zinc (mg/day)	11.5	11.7	11.7	11.7	0.64

*Egg consumption has been converted to grams and divided into quartiles (brackets present equivalent mean number of eggs consumed by participants in these quartiles)

†Proportion of persons consuming at least one serving of fish per week

‡Antioxidant amounts include both diet (energy-adjusted) and supplements

Table 4.6. Multivariate adjusted* associations between quartiles of egg consumption and the 10 year incidence of late age-related macular degeneration

Variable	Late age-related macular degeneration			
	No. at risk	Cases	Cumulative Incidence	Relative Risk (95%CI)
Quartiles of egg consumption (equivalent mean eggs/week)†				
Q1 (0 eggs/week)	532	25	6.0	1
Q2 (1 egg/week)	514	12	3.0	0.49 (0.23-1.04)
Q3 (3 eggs/week)	487	15	3.6	0.64 (0.31-1.33)
Q4 (6 eggs/week)	502	7	1.8	0.29 (0.12-0.70)
p trend				0.01
Egg consumption - top 25% compared with bottom 25%				0.26 (0.10-0.66)

*Adjusted for age, gender, mean arterial blood pressure, body-mass index, smoking, HDL-cholesterol, qualification level, presence of diabetes, past history of myocardial infarction or stroke, fish consumption and antioxidant intakes.

†Egg consumption has been converted to grams and divided into quartiles (brackets present equivalent mean number of eggs consumed by participants in these quartiles)

Table 4.7. Multivariate adjusted* associations between egg consumption and the 10 year incidence of late age-related macular degeneration lesions

Variable	Geographic Atrophy				Neovascular Degeneration			
	No. at risk	Cases	Cumulative Incidence	Relative Risk (95%CI)	No. at Risk	Cases	Cumulative Incidence	Relative Risk (95%CI)
Quartiles of egg consumption (equivalent mean eggs/week)†								
Q1 (0 eggs/week)	524	11	2.9	1	533	16	3.7	1
Q2 (1 egg/week)	509	3	0.8	0.27 (0.07-1.00)	515	10	2.4	0.74 (0.31-1.78)
Q3 (3 eggs/week)	481	7	1.8	0.51 (0.17-1.50)	488	8	1.8	0.65 (0.25-1.68)
Q4 (6 eggs/week)	500	3	0.7	0.21 (0.05-0.79)	504	4	1.0	0.34 (0.12-1.09)
p trend				0.03				0.07

*Adjusted for age, gender, mean arterial blood pressure, body-mass index, smoking, HDL-cholesterol, qualification level, presence of diabetes, past history of myocardial infarction or stroke, fish consumption and antioxidant intakes.

†Egg consumption has been converted to grams and divided into quartiles (brackets present equivalent mean number of eggs consumed by participants in these quartiles)

Part 5

Diet and Cataract

Chapter 10: Omega fatty acids and an aging marker: nuclear cataract incidence

Abstract

Objective: Cataract is the leading cause of reversible blindness. Animal studies have reported that intakes of polyunsaturated fatty acids may delay the formation of mature cataracts. We explored whether dietary polyunsaturated fat consumption reduced the 10-year incidence of age-related cataract.

Design: Prospective cohort study

Participants: Of 3654 participants aged 49+ years at baseline (1992-4), 2335 (75.1% of survivors) were re-examined after 5 years and 1952 (75.6% of survivors) after 10 years.

Setting: Population-based sampling from two adjacent postcodes, Sydney, Australia

Methods: Baseline dietary information was collected using a validated food frequency questionnaire. Dietary intakes were estimated using Australian Tables of Food Composition and a fatty acid supplement database.

Main Outcome Measures: Incident nuclear, cortical and posterior subcapsular cataract was graded from lens photographs following the Wisconsin Grading System.

Results: Of 3654 baseline participants, 2174 were included. After adjusting for age, gender, BMI, smoking, hypertension, presence of myopia, dark brown iris colour, intraocular pressure, use of inhaled corticosteroids and consumption of antioxidants (cortical models additionally adjusted for sun-related skin damage and nuclear models for intraocular pressure), there was a 40% reduction in the risk of developing nuclear and PSC cataract among participants in the highest compared to the lowest tertile of omega-3 fatty acids (p_{trend}=0.01 and p_{trend}=0.06, respectively) consumption. Higher consumption of long chain omega-3 fatty acids was associated with a 35% reduction in 10-year risk of nuclear cataract. Frequent consumption of fish (RR 0.75, 95% CI 0.54-1.04, p_{trend}=0.05) and nuts (RR 0.54, 95%CI 0.36-0.81, p_{trend}=0.003) was also associated with a reduced risk of nuclear cataract.

Conclusions: We found that dietary consumption of omega-3 fatty acids, particularly long chain omega-3 fatty acids, was associated with a reduced risk of developing nuclear cataract, a known ageing biomarker.

Introduction

Cataract is the leading cause of blindness worldwide.[373] In developed countries, this is largely reversible through effective cataract surgery, but in developing countries the burden of cataract blindness remains high. The most common form of cataract, nuclear cataract, has been proposed as a marker for ageing.[374] with multiple studies showing that age-related nuclear cataract is associated with long-term mortality.[375-379] A recent study showed that persons with 'successful ageing' or extraordinary longevity and preserved cognition had a reduced age-specific prevalence and lifetime cumulative incidence of age-related nuclear cataract.[374] Certain experimental treatments directed at the oxidant pathway, considered critical to the pathogenesis of cataract and ageing, have also been shown to impede development of both.[143;374] One such experimental treatment is long chain omega-3 fatty acids, shown to possess cardioprotective, anti-inflammatory and immune-modulatory properties.[167] Oxidant-related damage produces a decline in omega-3 fatty acid content in brain and cardiac cell membranes, contributing significantly to aging of the brain and heart.[170;171;380-383] Omega-3 fatty acid supplementation in animal models has been shown to reverse aspects of this age-related decline.[170;171;380-383]

Animal studies also suggest that intakes of polyunsaturated fatty acids could delay the formation of mature cataract.[384;385] Changes in the polyunsaturated fatty acid composition of lens membranes in cataractous lenses have also been documented in human studies.[168;169] Given the role of polyunsaturated fatty acids in caractogenesis, changes in dietary fat consumption could potentially alter the long-term risk of cataract as well as slowing the ageing process.

Only a few studies have investigated the relationship between dietary fat consumption and incident cataract, with conflicting findings.[155;157-159;163-165] Data from the baseline and 5-year

follow-up examinations of the Blue Mountains Eye Study previously demonstrated that the consumption of long chain fatty acids was associated with reduced prevalence or 5-year incidence of nuclear cataract.[158;159] In this study, we aimed to assess the relationship between baseline dietary polyunsaturated fat consumption, its food sources, and the 10-year incidence of cataract.

Materials and Methods

Study population

We conducted a population-based cohort study of vision, common eye diseases and other health outcomes in an urban, predominantly Caucasian population aged 49 years or older in Blue Mountains region, west of Sydney, Australia. At baseline in 1992-4, 3654 participants (82.4% response) were examined.[211;237] Participants were examined at 5-year intervals; 2335 (75.1% of survivors) at the second examination in 1997-9, and 1952 (76.5% of survivors) at the third in 2002-4. The study adhered to recommendations of the Helsinki Declaration and was approved by the University of Sydney and Sydney West Area Human Ethics Committees. Written, informed consent was obtained from all participants.

Cataract Assessment

At baseline, a standardised demographic and medical history was taken. Questions regarding prior diagnosis of cataract and details of cataract surgery were included. All participants underwent detailed eye examinations. Slit-lamp lens photographs were taken from each eye using Ektachrome 200 color film (Kodak, Rochester, NY) and an SL-7E photograph slit-lamp camera (Topcon, Tokyo, Japan) to assess presence of nuclear cataract. Retroillumination lens photographs were taken using a CT-R cataract camera (Neitz Instruments, Tokyo, Japan) to

assess presence of cortical and posterior subcapsular (PSC) cataract. At both 5- and 10-year follow-up visits, similar questionnaires were used to collect updated demographic and medical history data for the past 5 years. Participants were examined in approximately the same order as at baseline, using the same procedures and equipment.

Lens Grading

The Wisconsin Cataract Grading System, first developed in 1990 for use in the Beaver Dam Eye Study, was used to perform masked grading of the lens photographs. This method is well described.[239;240] A 5-point scale was used to assess the presence and severity of nuclear cataract, by direct comparison of participant photographs with 4 standard slit-lamp photographs of lenses with increasing opacity. Nuclear cataract was defined as nuclear opacity worse than standard 3. The presence and severity of cortical cataract and PSC were graded from Neitz photographs using a circular grid divided into 8 equal wedges and a central circle. Graders estimated the percentage area involved by cataract in each of the 9 segments. The opacity percentage in each segment was then summated to give a score for the whole lens area. Cortical cataract was considered present when the opacity involved at least 5% of the lens area. PSC cataract was defined if any was present. Intergrader reproducibility for baseline photographs was 0.79 for nuclear, 0.78 for cortical, and 0.65 for PSC cataract. The 10-year follow-up photographs were graded by one examiner for all 3 types of cataract, and the same examiner graded a random sample of baseline photographs to compare intergrader reproducibility. This gave weighted kappa 0.52 for nuclear, 0.72 for cortical, and 0.79 for PSC cataract. All positive cortical and PSC cataract cases were also graded by another senior grader. Nuclear and PSC cataract cases detected at all 3 examinations were either graded or re-graded by a senior researcher (JJW)

whose intragrader reliability was 0.79 for both nuclear and PSC cataract. Adjudication was provided by an ophthalmologist (PM).

Dietary Data

As part of the interview and examination, participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular from a Willett questionnaire,[213] that incorporated a nine-category frequency scale and standard portion size estimates. The FFQ had reasonable concurrent validity[214] when validated against 4-day weighed food records collected on three occasions in one year (n=79). The FFQ was found to show moderate to good agreement for ranking individuals according to their fat intakes, yielding correlation coefficients between 0.4-0.7 (total fat r=0.68, saturated fat r=0.67, monounsaturated fat r=0.54, polyunsaturated fat r=0.44) and correctly-classifying over 70% of people within one quintile for all types of fat.[214]

Dietary intakes were estimated using Australian Tables of Food Composition (NUTTAB90),[216] and a fatty acid supplement database.[218;219] Consumption of long chain omega-3 fatty acids was estimated by summating dietary intakes of eicosapentaenoic acid (EPA 20:5:n-3), docosapentaenoic acid (DPA 22:5n-3) and docosahexaenoic acid (DHA 22:6:n-3). We extracted separate data on the frequency of consuming any fish (any species plus method of preparation) and oily fish (specifically salmon, tuna and sardines). We also extracted data on the overall consumption of nuts, eggs and margarine.

Demographic, lifestyle and dietary variables

The interview and examination was used to collect information on risk factors for cataract including physician-diagnosed history of stroke or myocardial infarction, past use of oral and inhaled corticosteroids and lifestyle factors such as smoking. Examinations incorporated assessment of iris color, refractive status using subjective refraction and sun-related skin damage which was assessed by examination of arms, hands and face and was rated on a four-level scale (none, mild, moderate, and severe). A single measure of systolic and diastolic blood pressure using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Mean arterial blood pressure was defined as 0.33×systolic blood pressure +0.67×diastolic blood pressure. Body mass index (BMI) was calculated as weight(kg) /height $(m)^2$. Diabetes was defined either from past history of diabetes and current diabetes treatment, or from fasting plasma glucose levels ≥7.0mmol/L at examination, using the World Health Organization diabetes classification.[224] Fasting blood samples were processed on the same day for total cholesterol by the Institute of Clinical Pathology and Medical Research, Westmead Hospital.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis System (version 9, SAS Institute, Cary, NC). Ten-year person-specific cumulative incidence was calculated using the Kaplan–Meier (product-limit) method. Participants who did not have a particular type of cataract or cataract surgery in either eye at baseline were considered at risk of developing that type of cataract during the follow-up period. Those who did not have a particular cataract type at the 5-year follow-up examination and did not present for the 10-year follow-up were censored to the 5-year examination date.

We examined the association between baseline consumption of dietary polyunsaturated fatty acids and main food sources of these fatty acids and the 10-year incidence of nuclear, PSC and cortical cataract. Fatty acid (macronutrient) variables were adjusted for total energy intake using the Willett residual method.[241] Intakes were divided into tertiles for fatty acid variables. Food variables were assessed on consumption frequency of specific serving size (fish, nuts, eggs and margarine). Discrete logistic models (modified form of survival analysis) were used to assess relationships between dietary variables and incident cataract at either of the two follow-up time points. The following potential confounders were considered: age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intakes of antioxidants (lutein and zeaxanthin, vitamin C and E, beta-carotene, zinc). Sun-related skin damage was also included in models for cortical cataract and intraocular pressure was included in models for nuclear cataract. Relative risks (RR) and 95% confidence intervals (CI) are presented.

Results

At the 10-year follow-up examinations, 1103 (30.2%) of 3654 baseline participants had died, 375 (10.3%) moved from the study area, and 224 (6.1%) declined, leaving 1952 (76%) survivors who participated. Adding the 5-year follow-up examinations, there were 2464 participants with follow-up data available. Those lost to follow-up tended to be younger, to have lower socioeconomic status and to smoke, but were less likely to have coronary heart disease.

The FFQ was attempted and returned by 3267 baseline participants (89.4%), with 2897 (79.3% of 3654 participants) having sufficiently complete and plausible FFQ data for analysis. Subjects were excluded if over 12 questions were missing, if any page was blank, or if daily energy

intakes were <2500 kJ or >18,000 kJ.[187;214] Participants without usable FFQ data were more likely to be older (mean age 69.3 years vs. 65.3 years), to be female (15.8% of women vs. 13.5% of men) or current smokers (17.7% vs. 14.2%) than those with usable FFQs.

There were 2174 baseline participants (59.4%) who had reliable dietary assessments, gradable lens photographs, had not had cataract surgery performed on either eye at baseline and participated in at least one follow-up examination. After excluding those with nuclear cataract at baseline, 337 of 1094 (30.8%) persons at risk of nuclear cataract developed this cataract type over the 10-year period. Similarly, 117 of 1724 (6.9%) persons at risk of PSC cataract developed this cataract type, and 334 of 1535 (21.8%) persons at risk of cortical cataract developed cortical cataract. Of the 926 persons at risk of developing any type of cataract, 423 (45.7%) developed cataract over the 10-year period. The baseline study characteristics of persons developing cataract over 10 years compared to persons who did not develop any cataract, are shown in **Table 5.1**. Persons with incident cataract tended to be older, female, to have higher MABP, diabetes, a past history of stroke and sun-related skin damage.

Table 5.2 shows the relationships between tertiles of polyunsaturated fatty acids and the 10-year incidence of nuclear, PSC and cortical cataract, after adjusting for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, intra-ocular pressure, use of inhaled corticosteroids and intake of antioxidants (lutein and zeaxanthin, vitamin c and e, beta-carotene, zinc). Models for cortical cataract were additionally adjusted for sun-related skin damage and those for nuclear cataract were adjusted for intraocular pressure.

Subjects in the highest compared to the lowest tertile of consumption of dietary omega-3 fatty acids had an approximate 40% risk reduction for the development of nuclear (p_{trend}=0.01) and PSC cataract, though the latter was of borderline significance (p_{trend}=0.06). Higher consumption of long chain omega-3 fatty acids was associated with a 35% risk reduction for the development of nuclear, but not PSC cataract, over 10 years. Although there was a 30% reduction in risk for nuclear cataract among persons in the third tertile of omega-6 fatty acid consumption, this trend was of borderline significance (p_{trend}=0.06). The magnitude of the risk reduction did not change after additional adjustment for other types of fatty acids.

Table 5.3 shows important food sources for polyunsaturated fatty acids in relation to the 10-year incidence of cataract. Fish is one of the most important sources of omega-3 fatty acids (especially long chain omega-3 fatty acids, with the highest concentrations in oily fish). Regular (twice weekly) fish in the diet was associated with a reduced incidence of nuclear cataract (relative risk, RR, 0.75, 95% CI 0.54-1.04, p_{trend}=0.05), but increasing consumption of oily fish was not associated with a reduced risk of nuclear cataract (p_{trend}=0.29).

Nuts provide another source of fatty acids, including monounsaturated, omega-6 and omega-3 fatty acids. Regular (weekly), consumption of nuts was associated with a reduction in the risk of nuclear cataract (RR 0.54, CI 0.36-0.81, p_{trend}=0.003).

Discussion

Age-related cataract represents a significant public health burden. Any delay in the age at presentation of cataract could potentially reduce the burden of associated visual impairment and cataract surgery[386]. In this study, we found that participants whose baseline consumption of

omega-3 fatty acids, specifically long chain omega-3 fatty acids, was in the top tertile of intakes for this population, had a reduced long-term risk of developing nuclear cataract. Weekly consumption of nuts was also associated with a lower risk of developing nuclear cataract. Regular fish consumption, defined as at least twice per week at baseline, was associated with a significant reduction in the 10-year incidence of nuclear cataract. As both fish and some nuts are important sources of omega-3 fatty acids and fish, in particular, has a high content of long-chain fatty acids, our findings support the notion that these fatty acids provide benefits not only for the heart but also for the eye. Given that nuclear cataract is an ageing biomaker,[146;374;387] our findings also lend support to the anti-ageing properties of omega-3 fatty acids.[383]

Other than nuclear opacity, there was a suggestion of a reduced risk for PSC cataract associated with the consumption of omega-3 fatty acids, although corresponding food sources (e.g. fish) were not significantly linked to this finding. We found no association between dietary polyunsaturated fats or specific foods and the incidence of cortical cataract, suggesting that the associations observed appear to be relevant only for nuclear cataract.

Strengths of our study include its longitudinal (10-year follow-up) nature with a well defined population-based sample, with reasonable follow-up (around 75% of surviving participants were re-examined at both 5- and 10-years), together with cataract diagnosis that was based on detailed grading of lens photographs and shown to have high reproducibility. Photographic graders were also masked to subject characteristics, including nutritional data. Our study has several limitations. These include the possibility of confounding from unmeasured lifestyle and social factors and the relatively high number of participants (one in five) with missing FFQ data, which is unavoidable in older population samples. There was also a proportion of the sample

with missing nuclear photographs at baseline, which was caused by a random camera error. However, non-gradable lens photographs occurred non-differentially among subjects with and without cataract. As previously reported,[388] participants with and without gradable nuclear photographs were similar with respect to age, gender, smoking history, history of diabetes, hypertension, steroid use, and alcohol consumption. Although bias from the adoption of healthy behaviours among participants with high omega-3 fatty acid consumption cannot be ruled out, our dietary measurements were made (1992-94) before widespread knowledge about the potential benefits of omega-3 fatty acid consumption was made available to the general public. Finally, another limitation of our study is the use of a single blood pressure measurement, which may not accurately reflect a participant's 'usual' blood pressure over time.

Our findings confirm previous observations from our study and other longitudinal studies. In 5-year follow-up examinations of the Blue Mountains Eye Study, we reported an association between the consumption of long chain omega-3 fatty acids and a 40% reduction in incident nuclear cataract, but we could not previously link corresponding food sources with this finding.[159] Analyses of the full cohort of the Nurses Health Study (over 16 years), using cataract surgery as the outcome, demonstrated that women in the highest quintile of omega-3 fatty acid intakes or those who consumed fish more than three times per week, had a 12% lower risk of cataract extraction.[163] The findings were stronger in the group that reported nuclear cataract extraction.[163]

By contrast, analyses of the 5-year change in nuclear opacity of only 0.5% of the Nurses Health Study study sample[164] showed that alpha-linolenic acid (ALA), an omega-3 fatty acid, was associated with greater change in lens nuclear 'density'. The study was a smaller subset of the

entire Nurses Health Study cohort and had a relatively short follow-up period, which could have contributed to the discrepant results.

A role for polyunsaturated fatty acids in delaying the development of nuclear cataract is biologically plausible, although the exact mechanisms have not been fully investigated. Oxidation is thought to be crucial to the development of nuclear cataract.[146] A lens 'barrier' is thought to form in middle age, through the oxidation of lens proteins.[34] This virtually compartmentalises the lens, so that oxidised substances tend to remain in the nucleus, and antioxidants cannot enter the lens.[34] This is consistent with the finding that advanced nuclear cataract can occur without the lens cortex being affected. Oxidation of proteins can be triggered through many mechanisms. Changes in the lipid composition of the lens membrane can promote protein oxidation.[34;168;169]

Polyunsaturated fatty acids, in particular omega-3 fatty acids, are found in human lens membranes, and in cataractous human lenses their concentration is often decreased.[168;169] These nutrients play an important functional role, including improvement in membrane fluidity, enhancement of ligand-receptor function and activation of intracellular pathways.[170;171;381;383] High intakes of polyunsaturated fatty acids have shown to delay the formation of mature cataracts in experimental studies.[163;384;385] Restitution of lens membrane function may be a mechanism by which dietary omega-3 fatty acid intake reduces the risk of nuclear cataract. Furthermore, omega-3 fatty acids have been shown to have anti-oxidant properties.[167] In addition to the effect these anti-oxidant properties may have on caractogenesis, they have been shown to improve ageing related tissue decline. For example, supplementation with omega-3 fatty acids results in improvement in cell oxygen utilisation and calcium homeostasis in cardiac

cells and improved neural membrane fluidity and even induction of genes required for normal brain function.[170;171;380-383;389] Oral supplementation of micronutrients has also been reported to reduce the incidence of nuclear cataract, possibly through their antioxidant properties, although the exact micronutrients responsible are not known.[390]

In summary, this study has shown that a diet rich in omega-3 fatty acids, particularly long chain omega-3 fatty acids, is associated with a lower long-term risk of nuclear cataract, the most frequent form of age-related cataract. It did not reduce the incidence of the other major cataract types, PSC or cortical cataract. Particular foods (e.g. fish and nuts) rich in long-chain omega-3 fatty acids were also associated with a lower risk of nuclear cataract. As cataract represents a significant health and economic burden, dietary modification and publicity to increase the consumption of omega-3 fatty acids could be considered after further evidence from randomised clinical trials. As nuclear cataract is hypothesized to be an ageing marker, confirmation of these findings could improve our understanding of ageing processes and potentially lead to interventions that could assist in achieving the goal of healthy ageing.

Table 5.1. Baseline characteristics of persons with and without 10-year incident cataract*†

Characteristics	Incident cataract (n=423)	No cataract (n=503)	p-value
Age (yrs)	64.7	59.4	<0.001
Female gender (%)	49.3	50.7	<0.001
Mean arterial blood pressure (mmHg)	104.2	102.8	0.06
Body-mass index	26.6	26.2	0.18
Intraocular pressure (mmHg)	16.2	16.0	0.27
Current smoker (%)	14.4	13.4	0.64
Presence of diabetes mellitus (%)	6.2	3.8	0.09
History of coronary heart disease (%)	13.6	10.4	0.14
History of stroke (%)	4.5	1.6	0.009
Ever use of steroid inhalers (%)	10.4	9.4	0.85
Sun-related skin damage (Y/N)	27.3	18.4	0.001
Dark brown iris colour	9.2	7.6	0.35
Myopia (<-1 D)	12.7	15.3	0.09
Fasting serum cholesterol (mmol/L)	6.0	6.0	0.97
Consumption of fish (grams/day)	26.9	28.1	0.89
Vitamin E (mg/day)	8.14	8.11	0.36
Vitamin C (mg/day)	200.8	198.3	0.56
Beta-carotene (µg/day)	6685.5	6317.8	0.43
Zinc (mg/day)	11.8	11.5	0.09
Lutein & zeaxanthin (µg/day)	789.5	737.0	0.57

*Figures are based on persons without any cataract at baseline

†Data are expressed as means unless otherwise indicated

Table 5.2. Associations between tertiles of polyunsaturated fat consumption and 10-year incident cataract, after multivariable adjustment*

	Nuclear cataract			Posterior subcapsular cataract			Cortical cataract		
Variable (median grams)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Polyunsaturated fat									
Tertile 1 (8.7)	349	105	1	559	33	1	500	123	1
Tertile 2 (12.3)	373	133	1.25 (0.90-1.74)	589	50	1.61 (0.99-2.62)	529	113	0.82 (0.61-1.10)
Tertile 3 (16.6)	372	99	0.83 (0.59-1.17)	576	34	1.05 (0.62-1.78)	506	98	0.77 (0.57-1.04)
P (trend)			0.24			0.99			0.08
Omega 3 fatty acids									
Tertile 1 (0.6)	356	130	1	567	45	1	511	124	1
Tertile 2 (0.9)	378	116	0.77 (0.56-1.07)	590	42	0.82 (0.52-1.29)	522	108	0.85 (0.63-1.14)
Tertile 3 (1.2)	360	91	0.62 (0.44-0.88)	567	30	0.63 (0.38-1.04)	502	102	0.84 (0.62-1.14)
P (trend)			0.01			0.06			0.27
Long chain omega-3 fatty acids									
Tertile 1 (0.1)	361	111	1	563	39	1	499	94	1
Tertile 2 (0.2)	360	126	1.16 (0.84-1.62)	581	37	0.90 (0.55-1.48)	524	138	1.62 (1.20-2.20)
Tertile 3 (0.4)	373	100	0.65 (0.46-0.91)	602	41	1.06 (0.66-1.70)	512	102	1.17 (0.85-1.61)
P (trend)			0.004			0.78			0.86
Omega 6 fatty acids									
Tertile 1 (4.8)	343	125	1	558	45	1	489	107	1
Tertile 2 (6.9)	360	103	0.68 (0.49-0.96)	564	36	0.70 (0.43-1.14)	493	126	1.25 (0.93-1.70)
Tertile 3 (10.3)	391	109	0.70 (0.50-0.97)	602	36	0.80 (0.50-1.29)	543	99	0.87 (0.63-1.19)
P (trend)			0.06			0.38			0.20

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, intra-ocular pressure, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin C and E, beta-carotene, zinc). Cortical cataract models additionally adjusted for sun-related skin damage and nuclear cataract models adjusted for intraocular pressure.

Table 5.3. Associations between important food sources of polyunsaturated fatty acids and 10 year incident cataract, after multivariate adjustment*

	Nuclear			Posterior subcapsular cataract			Cortical		
Variable	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Total fish									
Less than once per week	427	141	1	686	38	1	609	223	1
Once per week	298	91	0.84 (0.60-1.18)	467	36	1.21 (0.90-1.64)	423	75	1.59 (0.97-2.62)
At least twice per week	362	103	0.75 (0.54-1.04)	563	41	1.11 (0.82-1.49)	493	34	1.38 (0.85-2.25)
P (trend)			0.05			0.47			0.19
Oily Fish									
Less than once per week	691	216	1	1104	73	1	983	223	1
Once per week	268	86	0.99 (0.72-1.35)	410	30	0.87 (0.65-1.18)	370	75	1.23 (0.76-1.98)
At least twice per week	128	33	0.62 (0.39-1.00)	202	12	0.86 (0.57-1.30)	172	34	1.30 (0.79-2.13)
P (trend)			0.29			0.33			0.30
Nuts									
Never	201	81	1	358	26	1	609	125	1
Less than once per week	553	171	0.74 (0.52-1.06)	861	63	0.95 (0.68-1.31)	423	98	1.14 (0.68-1.90)
At least once per week	331	83	0.54 (0.36-0.81)	491	27	0.95 (0.66-1.36)	493	109	0.87 (0.48-1.60)
P (trend)			0.003			0.98			0.60
Margarine									
Less than onche per week	197	81	1	302	91	1	402	23	1
1-7/week	186	86	0.97 (0.65-1.43)	309	93	1.60 (0.89-2.88)	424	36	0.93 (0.66-1.30)
At least twice per week	344	163	0.90 (0.64-1.28)	557	140	1.44 (0.84-2.48)	734	56	0.82 (0.60-1.11)
P (trend)			0.54			0.28			0.18

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, intra-ocular pressure, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin C and E, beta-carotene, zinc). Cortical cataract models additionally adjusted for sun-related skin damage and nuclear cataract models adjusted for intraocular pressure.

Table 5.3. continued*

Variable	Nuclear			Posterior subcapsular cataract			Cortical		
	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Eggs									
Less than once per week	213	58	1	349	21	1	1104	73	1
Once per week	433	133	1.06 (0.72-1.56)	679	51	0.91 (0.65-1.26)	410	30	1.17 (0.68-2.02)
Twice or more per week	36	9	1.47 (0.61-3.50)	53	3	0.67 (0.28-1.57)	202	12	0.80 (0.18-3.65)
P (trend)			0.52			0.37			0.78

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, intra-ocular pressure, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin C and E, beta-carotene, zinc). Cortical cataract models additionally adjusted for sun-related skin damage and nuclear cataract models adjusted for intraocular pressure.

Chapter 11 – Proteins, micronutrients and food sources, and the long-term incidence of cataract

Abstract

Objectives: Previous studies have suggested a relationship between protein consumption and incident cataract. We investigated the relationship between baseline dietary intakes of protein, nutrient components and major food sources of protein and the 10-year incidence of cataract.

Methods: Population-based cohort study with 3654 participants (49+ years) examined at baseline (1992-4), 2335 re-examined after 5 years and 1952 after 10 years. Incident nuclear, cortical and posterior subcapsular (PSC) cataract was graded from retinal photographs using the Wisconsin Grading System. Baseline dietary information was collected using a validated food frequency questionnaire.

Results: The baseline sample comprised 2174 participants. There was a reduction in the 10-year risk of nuclear cataract among participants in the 3rd and 4th quintiles of consumption of protein, and of PSC cataract for those in the 5th quintile of consumption, but no significant trends existed (nuclear p_{trend}=0.31, PSC p_{trend}=0.32). Higher consumption of vitamin B12 was associated with a progressively reduced risk of nuclear cataract (p_{trend}=0.0006). Higher quintiles of dietary riboflavin (p_{trend}=0.05) and calcium (p_{trend}=0.003) were also associated with a reduced risk of PSC cataract. Food sources of protein showed that consumption of legumes was also associated with a reduced risk of PSC cataract (5th quintile: 40% risk reduction p_{trend}=0.04).

Conclusions: Our findings suggest that a diet high in vitamin B12 could reduce the long-term risk of developing nuclear cataract, and a diet high in riboflavin, calcium or legumes (among persons consuming meat) may reduce the risk of incident PSC cataract. Replication of these findings is needed.

Introduction

Age-related cataract is the most frequent cause of visual impairment in older persons and represents a significant public health burden.[373] Delaying the age of onset of cataract could potentially reduce the burden of cataract surgery, and cataract-related visual impairment and blindness.[386] In developing countries, this is relevant, given the reported higher prevalence, more limited resources for surgery and greater size of rural populations.[150;391] Age-related cataract includes three common morphologic types, nuclear, cortical and posterior subcapsular (PSC).

Many studies,[122;132;139;140;150-162] including an interventional study,[122] have investigated the relationship between dietary protein consumption or micronutrients related to protein, and the incidence of cataract. Findings from some[122;150;158;159] but not all of these studies[151] have shown that a diet with overall low levels of protein consumption or malnutrition was associated with increased risk of cataract. Dietary riboflavin (possibly in conjunction with niacin and thiamin, since the three are highly correlated) has consistently been identified as an important nutrient related to the incidence of cataract.[122;134;139;140;152;154;155;161] This finding was supported by biochemical studies that assessed riboflavin associated enzyme activity in persons with and without cataract.[151;152;160]

Data from the baseline and 5-year follow-up examinations of the Blue Mountains Eye Study (BMES) did not provide entirely consistent findings on these relationships. The baseline study reported a decreased nuclear cataract prevalence in association with higher dietary consumption of protein, riboflavin, thiamin and niacin[158], whereas the 5-year longitudinal study reported a reduced incidence of PSC cataract in association with higher consumption of protein.[159] We also

assessed cross-sectional relationships between use of vitamin supplements and cataract, and found that use of thiamin and vitamin B12 supplements was associated with a reduced prevalence of both nuclear and cortical cataract.[154]

Given these inconsistent results from previous studies, we aimed, in the BMES population-based cohort, to assess whether higher baseline dietary intakes of proteins and nutrients predicted a reduced long-term (10-year) incidence of cataract. We also assessed whether specific food sources of significant protein were related to the long-term incidence of cataract.

Materials and Methods

Study population

We conducted a population-based cohort study of vision, common eye diseases and other health outcomes in the urban, predominantly Caucasian population aged 49 years or older, who resided in the Blue Mountains region, west of Sydney, Australia. At baseline in 1992-4, 3654 participants (82.4% response) were examined.[211;237] Follow-up examinations were conducted at 5-year intervals; 2335 (75.1% of survivors) were examined at the 5-year follow-up in 1997-9, and 1,952 (76.5% of survivors) at the 10-year follow-up in 2002-4. The study adhered to recommendations of the Helsinki Declaration and was approved by the University of Sydney and Sydney West Area Human Ethics Committees. Written, informed consent was obtained from all participants at each examination.

Cataract Assessment

At baseline, a standardised demographic and medical history was taken. Questions regarding a previous diagnosis of cataract and details of cataract surgery were included, and participants underwent detailed eye examinations. Lens photographs were taken of each eye using Ektachrome 200 color film (Kodak, Rochester, NY). An SL-7E camera (Topcon, Tokyo, Japan) was used to document nuclear opacity on slit-lamp photographs, while a CT-R cataract camera (Neitz Instruments, Tokyo, Japan) was used to document cortical and PSC cataract on retroillumination lens photographs. At the 5- and 10-year follow-up visits, similar questionnaires were used to collect updated demographic data and medical history information over the previous 5 years. Participants were examined in approximately the same order as at baseline, using the same procedures and equipment.

Lens Grading

The Wisconsin Cataract Grading System, first developed in 1990 for use in the Beaver Dam Eye Study, was used to perform masked grading of the lens photographs. Details of this method are reported.[239;240] A 5-point scale was used to assess the presence and severity of nuclear cataract, by directly comparing participant photographs with 4 slit-lamp photograph standards of increasing lens opacity. Nuclear cataract was defined as nuclear opacity worse than standard 3. The presence and severity of cortical cataract and PSC were graded from the Neitz photographs using a circular grid divided into 8 equal wedges and a central circle. Graders estimated the percentage area in each of the 9 segments involved by cataract. The percentage of opacities in each segment was then summated to give a score for the whole lens area. Cortical cataract was considered present when the opacity involved at least 5% of the lens area. PSC cataract was defined if any was present. The inter-grader reproducibility for the baseline lens photograph

grading was 0.79 for nuclear cataract, 0.78 for cortical cataract, and 0.57 for PSC cataract. After excluding 2 eyes with co-existing dense nuclear cataract, the weighted kappa for PSC cataract improved to 0.65. The 10-year follow-up photographs were graded by one examiner (GK) for all 3 cataract types The same examiner graded a random sample of baseline photographs to compare inter-grader reproducibility, giving a weighted kappa of 0.52 for nuclear cataract, 0.72 for cortical cataract, and 0.79 for PSC cataract. All positive cortical cataract cases were adjudicated by another senior grader (AGT), while nuclear and PSC cataract cases detected at all 3 examinations were either graded or re-graded by a senior researcher (JJW), whose intra-grader reproducibility was 0.79 for both nuclear and PSC cataract. Final adjudication was provided by a senior ophthalmologist (PM).

Dietary and Supplement Data

As part of the interview and examination, participants were asked to complete a detailed (145-item) food frequency questionnaire (FFQ). This was modified for Australian diet and vernacular from a Willett questionnaire,[213] that incorporated a nine-category frequency scale and standard portion size estimates. The FFQ had reasonable concurrent validity[214] when validated against 4-day weighed food records collected on three occasions over a one-year period (n=79). Correlation coefficients (r) comparing the FFQ and weighed food records were over 0.50 for all nutrients except protein (r=0.16), vitamin A (r=0.32) and iron(r=0.37). Questions were asked about specific foods and supplements. Dietary supplements were coded using supplement information reported in the Australian Register of the Therapeutic Goods Administration.[215] Dietary intakes of particular nutrients were estimated using the Australian Tables of Food Composition (NUTTAB90),[216] and both fatty acid and supplement databases.[218;219]

Demographic, lifestyle and dietary variables

The interview and examination was used to collect information on risk factors for cataract including physician-diagnosed history of stroke or myocardial infarction, past use of oral and inhaled corticosteroids and lifestyle factors such as smoking. The examination incorporated an assessment of iris color using photographic standards, refractive status using subjective refraction and sun-related skin damage, which was assessed by examining the arms, hands and face, and rated on a four-level scale (none, mild, moderate, and severe). A single measure of systolic and diastolic blood pressure using a mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Mean arterial blood pressure was defined as 0.33×systolic blood pressure +0.67×diastolic blood pressure. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Diabetes was defined either from past history of diabetes and current diabetes treatment, or from fasting plasma glucose levels ≥7.0mmol/L at examination, using the World Health Organization diabetes classification.[224] Fasting blood samples were processed the same day for total cholesterol by the Institute of Clinical Pathology and Medical Research, Westmead Hospital.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis System (version 9, SAS Institute, Cary, NC). Ten-year person-specific cumulative incidence was calculated using the Kaplan–Meier (product-limit) method. Participants who did not have a particular type of cataract or cataract surgery in either eye at baseline were considered at risk of developing that type of cataract during the follow-up period. Those who did not have a particular cataract type at the 5-year follow-up examination and who did not return to 10-year follow-up examinations were censored to the 5-year examination date.

We examined the association between baseline consumption of protein and the main food sources of protein and the 10-year incidence of nuclear, cortical and PSC cataract. Protein and micronutrient variables were adjusted for total energy intake using the Willett residual method.[241] Subject intakes were divided into quintiles for protein and food variables (e.g. meats including red meats, eggs, poultry, legumes and dairy foods). Discrete linear logistic modeling (a modified form of survival analysis) was used to assess relationships between dietary variables and incident cataract at either of the two follow-up time points. The following potential confounders were considered: age, gender, body mass index (BMI), smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamins C and E, beta-carotene and zinc, as these have been associated with cataract in the BMES). The presence of sun-related skin damage was also included in models for cortical cataract, and intraocular pressure was included in models for nuclear cataract. Relative risks (RR) and 95% confidence intervals (CI) are presented.

Results

The baseline cohort consisted of 3654 participants. At the 10-year follow-up examinations, 1103 (30.2% of original cohort) of participants had died, 375 (10.3%) had moved from the study area, and 224 (6.1%) refused to participate, leaving 1952 (76%) surviving participants. Together with the 5-year follow-up examinations, there were 2464 participants with follow-up data available. Those lost to follow-up tended to be younger, to have lower socioeconomic status and to smoke, but were less likely to have coronary heart disease.

The FFQ was attempted and returned by 3267 baseline participants (89.4%), with 2897 (79.3% of total participants) having sufficiently complete and plausible FFQ data for analysis. Subjects

were excluded if over 12 questions were missing, if an entire page remained blank, or if daily energy intakes were <2500 kJ or >18,000 kJ.[187;214] Participants without usable FFQ data were more likely to be older (mean age 69.3 years vs. 65.3 years), female (15.8% of women vs. 13.5% of men) or current smokers (17.7% vs. 14.2%) than those with usable FFQs.

Of the 2464 baseline study participants who had been followed at least once, 2174 participants (88.2%) had reliable dietary assessment, gradable lens photographs, and had not had cataract surgery performed on either eye at baseline and had participated in at least one follow-up examination; they were included in the current analyses. After excluding persons with nuclear cataract at baseline, over the 10-year period, 337 of 1094 (30.8%) persons at risk developed nuclear cataract. A total 117 persons of 1724 (6.9%) at risk developed PSC and 334 persons of 1535 (21.8%) at risk developed cortical cataract. There were 926 persons at risk of any type of cataract, and of these, 423 (45.7%) developed some cataract over the 10-year period. The baseline characteristics of persons who developed any cataract over this time compared with persons who did not develop cataract are shown in **Table 5.4**. Persons with 10-year incident cataract tended to be older, female, to have higher MABP, diabetes, a past history of stroke and sun-related skin damage.

Table 5.5 shows multivariable-adjusted associations between quintiles of protein consumption and the 10-year incidence of nuclear, PSC and cortical cataract. For incident nuclear cataract, there was a significant reduced risk among persons in the 3rd (RR 0.54, CI 0.35 to 0.83) and 4th (RR 0.57, CI 0.37 to 0.88) quintile of protein consumption, but there was no significant trend in this association (p_{trend}=0.31). Participants in the highest quintile of protein consumption had the lowest risk of PSC cataract (RR 0.49, CI 0.26 to 0.95), but again the trend was non-significant

(p_{trend}=0.32). No associations were found between protein consumption and incident cortical cataract.

Table 5.6 shows a similar analysis for the micronutrients commonly associated with protein-containing foods and 10-year incident cataract. There was a significant, protective association between intakes of vitamin B12 (incorporating B12 from foods and supplements) and the 10-year incidence of nuclear cataract (5^{th} quintile, RR, 0.44, 95% CI, 0.28 to 0.72, p_{trend}=0.006). In contrast, intakes of riboflavin and its correlated B-group vitamins, thiamin and niacin, were not related to nuclear cataract incidence. Intakes of riboflavin (5^{th} quintile, RR, 0.64, 95% CI, 0.31 to 1.34, p_{trend}=0.03) and calcium (5^{th} quintile, RR, 0.28, 95% CI, 0.07 to 1.16, p_{trend}=0.05) were inversely associated with incident PSC cataract. No associations between these micronutrients and cortical cataract incidence were found.

Table 5.7 shows the main food sources of protein in relation to the 10-year incidence of cataract. Consumption of legumes, an important source of protein, particularly for vegetarians, was associated with a reduced risk of PSC cataract (RR 0.60, CI 0.29 to 1.20, p_{trend}=0.04). Significant associations were not found between the consumption of other food types and incidence of any type of cataract.

We also investigated interactions of age or smoking status with the nutrients assessed, but found no significant interactions.

Discussion

In this longitudinal study, we found that a diet high in vitamin B12 (predominantly from food sources) was associated with a reduced long-term risk of developing nuclear cataract, the most frequent type of age-related cataract. Our findings also confirm earlier data showing that a diet high in riboflavin and calcium, micronutrients commonly found in protein-rich foods, is associated with a reduced risk of incident PSC cataract. In contrast, we could not confirm significant protective associations of riboflavin, thiamin and niacin with nuclear cataract that were demonstrated previously in our study sample.[154;158] We also found that that higher consumption of legumes was associated with a reduced risk of PSC cataract, a finding not previously reported. These protective associations with nuclear or PSC cataract incidence were independent of the principal known cataract risk factors and dietary constituents, including vitamin C and other antioxidants, previously reported to be associated with cataract. We also could not demonstrate associations between any of the dietary factors examined and incident cortical cataract.

Many previous studies have examined the association of cataract with protein consumption and the B-group vitamins, riboflavin and niacin. However, no studies have reported the longitudinal association of cataract with dietary intakes of vitamin B12. In the Blue Mountains Eye Study baseline survey, we reported an association between consumption of vitamin B12 in supplement form and a reduced prevalence of nuclear and cortical cataract.[154] Stratification by smoking status in the study sample suggested a stronger protective association with multivitamin supplement use among smokers.[154] Using the longitudinal data from the same study sample, we are able to confirm the protective association of these nutrients with a reduced risk of nuclear, but not cortical cataract. Furthermore, careful examination of vitamin B12 consumption

suggested that the consumption of vitamin B12 in the study sample was predominantly from whole food items rather than supplements. The protective association was not modified by smoking status in the incidence data. As indicated from the Beaver Dam Eye Study findings,[156] dietary intakes are likely to have long-term impacts on the risk of developing cataract, rather than on the concurrent prevalence of this condition.

A recent trial showed that oral supplementation of micronutrients (including vitamin B12) reduced the incidence of nuclear cataract, although since there was a mixture of micronutrients, the exact nutrients responsible are not known.[390]

Our finding of an association between the vitamin B12 intake and cataract is consistent with previous findings from an Indian study that documented non-significantly lower levels of serum vitamin B12 and significantly higher levels of serum homocysteine in cataract cases compared with controls.[392] A relationship of vitamin B12 with cataract is also biologically plausible. Vitamin B12 and folate are critical to the metabolism of homocysteine, which, at high levels, may lead to oxidative stress via the spontaneous production of reactive oxidative species.[392-395] Homocysteine has been found to be present in the aqueous fluid surrounding the lens.[396;397] Oxidative damage is thought to be a critical mechanism for cataract formation,[146] and oxidized amino acids have been found to accumulate in the lens.[34;146;398] Oxidation of lens amino acids may result in altered interactions between protein and water molecules, thus contributing to lens opacification.[146] In nuclear cataract, a lens 'barrier' is thought to form in middle age via the lens protein oxidation, [34;146] which virtually compartmentalizes the lens, so that oxidised substances penetrate and remain within the nucleus, but antioxidants cannot enter.[34;146]

The baseline and 5-year follow-up Blue Mountains studies reported that higher levels of protein consumption were associated with a reduced prevalence of nuclear cataract or reduced incidence of PSC cataract, respectively.[158;159] Although these baseline and 5-year follow-up findings were somewhat supported by the 10-year follow-up data in our current report, with the highest quintile of protein consumption associated with a reduced risk of PSC cataract, we could not find a significant trend in this association. Two studies conducted in likely malnourished Indian populations[150;151] showed a strong association between any cataract or previous surgery and low levels of protein consumption. However, in two developed country populations[155;162], no associations were found between dietary protein and any category of cataract.

A US study that examined components and products of protein, reported a lower risk of cataract in persons with higher serum levels of iron, albumin-globulin ratio and glycine and aspartic amino acids[152]. Serum levels of iron, albumin, and amino acids, however, may not necessarily be well correlated with dietary intakes (and similarly may not correlate well with the aqueous levels of these nutrients).

Most of the evidence to date consistently supports the association of high riboflavin consumption with a reduced risk of various types of cataract, but overwhelmingly for nuclear cataract.[122;151-156;158] In contrast, we could not demonstrate an association between riboflavin intakes and incident nuclear cataract, but could with PSC cataract, an association only a few studies have documented.[151;152] The reasons for these discrepant findings in the different studies are unclear, though may relate to variability in demographic characteristics, environmental exposures and overall dietary patterns across studies. Importantly, the inconsistency in study findings could suggest that the observed associations may not be real and could be due to

residual confounding effects. In addition, the limited study power from any single study could also explain the inconsistency. If the association with cataract were only modest, a relatively small number of cases (such as PSC cataract) in a single study could substantially affect the magnitude of the association. However, the dietary riboflavin and cataract association is strengthened by biochemical findings from many studies[151;152;160;162] that also report an inverse association between riboflavin status in erythrocytes and cataract.

We also could not demonstrate an association between intakes of thiamin or niacin and the incidence of any cataract type. As these latter two nutrients are highly correlated with riboflavin consumption, other studies reporting protective associations with these nutrients, could potentially be explained by the correlation with riboflavin, consistently found to be associated with incident cataract.[158]

Our study is the first conducted in a developed country to show an association between higher legume consumption and reduced risk of PSC cataract. Increased odds for presence of any cataract was reported from an Indian study in relation to diets low in lentils (a form of legume).[150] Legumes are recognised to not only contain protein but also fibre, riboflavin and isoflavone. They are also thought to have an advantage in terms of calcium retention in the body.[399] The riboflavin and calcium content of legumes could explain our findings. A protective role for calcium in relation to cataract was found by Tavani et al[157] and calcium is an important component in food sources of protein such as dairy food.

Strengths of our study include its 10-year longitudinal data collection from a population-based sample, reasonable follow-up rates (over 75% of surviving participants were re-examined), and

cataract diagnosis based on a detailed grading of lens photographs, found to be highly reproducible. The graders were also masked to subject characteristics, including nutritional data. Our study has many limitations, including the possibility of confounding from unmeasured lifestyle and other factors, and the relatively high proportion of participants with missing FFQ data, which may be unavoidable in older populations. A higher proportion of participants had missing nuclear photographs at baseline, due to a random camera error, than for the other two cataract types. However, participants with ungradable lens photographs were non-differentially distributed among subjects with and without cataract and participants with and without gradable photographs were also similar with respect to smoking history, age, gender, history of diabetes, hypertension, steroid use, and alcohol intake.[388] There is the possibility of confounding by healthy behaviours, often associated with higher levels of multivitamin intake or a higher consumption of protein among persons conscientious about their health, such as protection from excessive sun exposure. However, sun exposure has mostly been linked (weakly) to cortical cataract and we could not document any protective association of dietary nutrients and the risk of cortical cataract. Since we collected dietary data long before cataract was diagnosed, indication bias is also unlikely. Dietary nutrients are consumed together with other nutrients and food items, so that examining nutrients in isolation may not be the best way to study associations with diet. Another limitation of our study is the use of a single blood pressure measurement, which may not accurately reflect a participant's 'usual' blood pressure over time. Finally, the relatively small numbers of subjects who were at risk of cataract, indicate that confirmation of our study findings in future studies will be needed.

In summary, we showed in this predominantly Caucasian population, that a diet high in vitamin B12 may be associated with a reduced long- term risk of developing nuclear cataract, but not

PSC or cortical cataract, while a diet high in riboflavin and calcium was associated with a reduced risk of PSC cataract, but not nuclear or cortical cataract. We also found that higher consumption of legumes may also be associated with a reduced risk of PSC cataract, despite the relatively high consumption of meat protein. Our study findings need to be confirmed in other study samples. The use of different study approaches, such as examination of the global effects of diet patterns or scores, is also needed.

Since cataract represents a significant health care burden, particularly in developing countries, our study findings, if confirmed, could reinforce the message that our daily lifestyle and diet patterns may play an important role in the development or prevention of common diseases, and that preventive strategies could start with modification of daily behaviours such as dietary or lifestyle routines.

Table 5.4. Baseline characteristics of persons with and without 10-year incident cataract*†

Characteristics	10-year incident cataract (n=423)	No incident cataract (n=503)	p-value
Age (yrs)	64.7	59.4	<0.001
Female gender (%)	49.3	50.7	<0.001
Mean arterial blood pressure (mmHg)	104.2	102.8	0.06
Body-mass index	26.6	26.2	0.18
Intraocular pressure (mmHg)	16.2	16.0	0.27
Current smoker (%)	14.4	13.4	0.64
Presence of diabetes mellitus (%)	6.2	3.8	0.09
History of coronary heart disease (%)	13.6	10.4	0.14
History of stroke (%)	4.5	1.6	0.009
Ever use of steroid inhalers (%)	10.4	9.4	0.85
Sun-related skin damage (Y/N)	27.3	18.4	0.001
Dark brown iris colour	9.2	7.6	0.35
Myopia (<-1 D)	12.7	15.3	0.09
Fasting serum cholesterol (mmol/L)	6.0	6.0	0.97
Consumption of fish (grams/day)	26.9	28.1	0.89
Vitamin E (mg/day)	8.14	8.11	0.36
Vitamin C (mg/day)	200.8	198.3	0.56
Betacarotene (µg/day)	11.8	11.5	0.09
Zinc (mg/day)	6685.5	6317.8	0.57
Lutein & zeaxanthin (µg/day)	789.5	737.0	0.43

*Figures are based on persons without any cataract at baseline

†Data are expressed as means unless otherwise indicated

Table 5.5. Associations between quintiles of protein consumption and 10-year incident cataract, after multivariable adjustment*

Variable (median grams)	Nuclear cataract			Posterior subcapsular cataract			Cortical cataract		
	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Protein									
Quintile 1	202	70	1	331	24	1	294	60	1
Quintile 2	215	65	0.70 (0.45-1.08)	324	18	0.57 (0.30-1.11)	292	49	0.89 (0.58-1.35)
Quintile 3	231	64	0.54 (0.35-0.83)	351	27	0.79 (0.44-1.42)	315	70	1.09 (0.73-1.61)
Quintile 4	230	66	0.57 (0.37-0.88)	368	28	0.75 (0.42-1.35)	328	82	1.14 (0.78-1.69)
Quintile 5	216	72	0.76 (0.49-1.17)	350	20	0.49 (0.26-0.95)	306	73	1.16 (0.78-1.73)
P (trend)			0.31			0.32			0.21

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin c and e, beta-carotene, zinc). Cortical cataract models also adjusted for sun-related skin damage and nuclear cataract models also adjusted for intraocular pressure.

Table 5.6. Associations between major protein-associated vitamins and 10 year incident cataract, after multivariate adjustment*

Variable	Nuclear			PSC			Cortical		
	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Vitamin B12 (micrograms)									
Quintile 1	201	70	1	330	24	1	287	53	1
Quintile 2	205	60	0.56 (0.36-0.88)	335	25	0.82 (0.45-1.49)	293	65	1.06 (0.70-1.60)
Quintile 3	225	76	0.71 (0.46-1.08)	336	31	1.04 (0.58-1.85)	304	71	1.36 (0.90-2.03)
Quintile 4	238	81	0.71 (0.46-1.08)	359	14	0.40 (0.19-0.81)	320	78	1.25 (0.84-1.87)
Quintile 5	225	50	0.44 (0.28-0.72)	364	23	0.70 (0.38-1.31)	331	67	1.26 (0.83-1.91)
P (trend)			0.006			0.44			0.81
Niacin (median milligrams)									
Quintile 1	187	59	1	326	20	1	274	58	1
Quintile 2	223	67	0.86 (0.55-1.35)	330	33	1.58 (0.86-2.90)	290	59	0.95 (0.03-1.44)
Quintile 3	227	78	0.96 (0.62-1.48)	361	21	0.89 (0.46-1.73)	327	82	1.19 (0.81-1.76)
Quintile 4	232	67	1.00 (0.64-1.57)	357	20	0.87 (0.44-1.71)	329	70	1.05 (0.70-1.57)
Quintile 5	225	66	0.92 (0.57-1.48)	350	23	1.08 (0.55-2.13)	315	65	1.10 (0.73-1.68)
P (trend)			0.45			0.77			0.70
Riboflavin (median milligrams)									
Quintile 1	185	64	1	312	19	1	264	58	1
Quintile 2	222	70	0.81 (0.52-1.25)	339	35	1.77 (0.96-3.28)	312	71	1.05 (0.71-1.57)
Quintile 3	220	60	0.65 (0.41-1.02)	351	26	1.25 (0.65-2.40)	299	56	0.88 (0.56-1.34)
Quintile 4	230	78	0.94 (0.61-1.45)	359	21	0.96 (0.49-1.90)	331	82	1.14 (0.77-1.70)
Quintile 5	237	65	0.70 (0.44-1.11)	363	16	0.64 (0.31-1.34)	329	67	1.00 (0.66-1.51)
P (trend)			0.16			0.03			0.61

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin c and e, beta-carotene, zinc). Cortical cataract models also adjusted for sun-related skin damage and nuclear cataract models also adjusted for intraocular pressure.

Table 5.6. continued.*

Variable	Nuclear			PSC			Cortical		
	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Thiamin (median milligrams)									
Quintile 1	88	53	1	135	9	1	119	18	1
Quintile 2	76	72	1.27 (0.64-2.55)	134	12	1.28 (0.51-3.24)	119	27	1.60 (0.80-3.18)
Quintile 3	89	80	1.10 (0.57-2.13)	132	6	0.64 (0.22-1.92)	115	34	2.31 (1.17-4.59)
Quintile 4	84	57	0.96 (0.47-1.96)	136	10	1.07 (0.41-2.77)	122	23	1.51 (0.75-3.05)
Quintile 5	93	75	0.69 (0.33-1.44)	135	6	0.49 (0.15-1.56)	129	26	1.75 (0.87-3.51)
P (trend)			0.10			0.41			0.84
Iron (median milligrams)									
Quintile 1	229	67	1	342	19	1	304	60	1
Quintile 2	216	72	0.97 (0.63-1.48)	337	25	1.25 (0.65-2.41)	312	71	1.16 (0.78-1.72)
Quintile 3	220	65	0.72 (0.47-1.12)	358	30	1.35 (0.71-2.57)	315	64	0.98 (0.65-1.49)
Quintile 4	214	65	0.67 (0.43-1.04)	339	26	1.23 (0.64-2.34)	299	63	0.94 (0.62-1.43)
Quintile 5	215	68	0.89 (0.57-1.38)	348	17	0.71 (0.34-1.50)	305	76	1.38 (0.92-2.07)
P (trend)			0.41			0.24			0.41
Calcium (median milligrams)									
Quintile 1	72	21	1	116	7	1	109	20	1
Quintile 2	90	32	0.73 (0.35-1.51)	141	10	**0.98 (0.35-2.74)**	122	26	1.04 (0.53-2.02)
Quintile 3	88	23	0.62 (0.28-1.35)	136	12	**1.57 (0.58-4.28)**	124	28	1.30 (0.67-2.53)
Quintile 4	94	29	0.85 (0.41-1.76)	156	11	**0.85 (0.30-2.38)**	138	29	0.94 (0.49-1.82)
Quintile 5	86	26	0.61 (0.29-1.32)	123	3	**0.28 (0.07-1.16)**	111	25	0.87 (0.42-1.74)
P (trend)			0.58			**0.05**			0.54

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin c and e, beta-carotene, zinc). Cortical cataract models also adjusted for sun-related skin damage and nuclear cataract models also adjusted for intraocular pressure.

Table 5.7. Associations between major food sources of protein and 10 year incident cataract, after multivariate adjustment*

Variable	Nuclear			PSC			Cortical		
	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Meat (median grams)									
Quintile 1	208	64	1	343	23	1	312	61	1
Quintile 2	249	77	0.90 (0.59-1.36)	373	28	0.94 (0.52-1.71)	322	66	1.10 (0.74-1.62)
Quintile 3	202	67	1.21 (0.78-1.88)	336	27	1.13 (0.62-2.03)	309	70	1.19 (0.81-1.75)
Quintile 4	215	67	1.07 (0.69-1.64)	351	19	0.69 (0.36-1.30)	308	75	1.12 (0.76-1.65)
Quintile 5	223	62	0.85 (0.54-1.34)	324	20	0.74 (0.38-1.43)	285	62	1.18 (0.78-1.78)
P (trend)			0.66			0.50			0.22
Lean red meat (median grams)									
Quintile 1	213	63	1	342	25	1	302	57	1
Quintile 2	226	71	1.02 (0.67-1.57)	359	23	0.81 (0.44-1.49)	318	65	1.07 (0.72-1.60)
Quintile 3	223	74	1.27 (0.67-1.57)	351	26	0.89 (0.49-1.62)	316	74	1.21 (0.81-1.79)
Quintile 4	213	63	1.15 (0.74-1.78)	341	23	0.89 (0.49-1.62)	304	70	1.15 (0.76-1.72)
Quintile 5	222	66	0.97 (0.61-1.52)	334	20	0.63 (0.32-1.23)	296	68	1.24 (0.82-1.88)
P (trend)			0.89			0.59			0.17
Eggs (median grams)									
Quintile 1	230	68	1	372	26	1	328	79	1
Quintile 2	224	79	1.36 (0.90-2.05)	350	17	0.63 (0.32-1.21)	315	69	1.10 (0.76-1.60)
Quintile 3	203	51	0.71 (0.45-1.12)	340	30	1.24 (0.71-2.16)	296	61	0.93 (0.63-1.36)
Quintile 4	215	65	0.99 (0.65-2.53)	342	27	1.00 (0.56-1.80)	330	66	0.93 (0.64-1.35)
Quintile 5	225	74	1.37 (0.90-2.10)	323	17	0.64 (-.33-1.27)	332	59	0.91 (0.61-1.35)
P (trend)			0.40			0.78			0.62

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin c and e, beta-carotene, zinc). Cortical cataract models also adjusted for sun-related skin damage and nuclear cataract models also adjusted for intraocular pressure.

Table 5.7. continued.*

Variable	Nuclear			PSC			Cortical		
	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)	No. risk	Cases	Relative Risk (95%CI)
Poultry (median grams)									
Quintile 1	270	83	1	431	32	1	373	85	1
Quintile 2	48	11	0.86 (0.41-1.77)	88	4	0.75 (0.26-2.19)	76	14	0.77 (0.40-1.50)
Quintile 3	317	108	1.20 (0.83-1.77)	527	36	0.90 (0.53-1.52)	477	101	0.89 (0.64-1.24)
Quintile 4	315	91	0.94 (0.64-1.38)	463	28	0.80 (0.47-1.38)	420	96	1.05 (0.75-1.47)
Quintile 5	147	44	1.30 (0.82-2.05)	218	17	1.13 (0.60-2.14)	190	38	0.91 (0.58-1.41)
P (trend)			0.87			0.92			0.69
Legumes (median grams)									
Quintile 1	161	56	1	277	22	1	251	67	1
Quintile 2	259	84	1.12 (0.72-1.73)	403	32	1.07 (0.59-1.93)	355	74	0.94 (0.64-1.38)
Quintile 3	244	69	0.76 (0.49-1.20)	399	27	0.87 (0.47-1.61)	353	70	0.85 (0.58-1.25)
Quintile 4	213	57	0.78 (0.49-1.26)	307	22	1.08 (0.56-2.06)	273	59	1.01 (0.67-1.51)
Quintile 5	220	71	1.03 (0.65-1.62)	341	14	0.60 (0.29-1.20)	304	64	0.89 (0.59-1.33)
P (trend)			0.78			0.04			0.91
Dairy (median grams)									
Quintile 1	204	68	1	326	25	1	286	65	1
Quintile 2	209	59	0.60 (0.38-0.95)	336	22	0.90 (0.49-1.65)	292	61	0.82 (0.55-1.23)
Quintile 3	226	71	0.75 (0.49-1.16)	336	20	0.78 (0.42-1.47)	296	69	1.06 (0.73-1.56)
Quintile 4	225	72	0.80 (0.52-1.22)	365	30	0.94 (0.53-1.68)	330	63	0.76 (0.51-1.12)
Quintile 5	233	67	0.65 (0.42-1.00)	364	20	0.55 (0.28-1.06)	332	76	0.90 (0.61-1.31)
P (trend)			0.22			0.23			0.81

*Adjusted for age, gender, body-mass index, smoking, hypertension, presence of myopia, dark brown iris colour, use of inhaled corticosteroids, intake of antioxidants (lutein and zeaxanthin, vitamin c and e, beta-carotene, zinc). Cortical cataract models also adjusted for sun-related skin damage and nuclear cataract models also adjusted for intraocular pressure.

Part 6

Diet and Visual Impairment

Chapter 12: Healthy dietary and lifestyle factors protect against incident visual impairment

Abstract

Background: There have been no data on whether dietary patterns are associated with the risk of developing visual impairment in older people.

Objective: To examine the association between the Healthy Eating Index (HEI) and the 10-year incidence of visual impairment.

Design: Population-based cohort study with 3,654 participants (49+ years) examined at baseline (1992-4), 2,335 re-examined after 5 years and 1,952 after 10 years. 10-year incident visual impairment was defined as visual acuity less than 6/12 at follow-up examinations among those with normal vision at baseline. The HEI provides a proportionate score for each participant from their baseline dietary intake and non-dietary lifestyle factors such as physical activity. The higher the score, the healthier the diet and lifestyle.

Results: Of 1963 persons, 244 (12.4%) developed unilateral visual impairment over 10 years. Cataract and age-related macular degeneration represented the two commonest causes of visual impairment. After adjusting for age, smoking and other risk factors, higher HEI score was associated with an 40% reduction in the risk of visual impairment (4^{th} quartile vs. 1^{st}: relative risk, RR, 0.60, 95% confidence interval, CI, 0.40 - 0.89, p_{trend}=0.016). There was a significant age interaction (p = 0.03), showing that persons aged ≥70 years and in the highest quartile of HEI had a significant reduction in the risk of visual impairment (4^{th} vs. 1^{st}: RR, 0.49, 95% CI, 0.27 – 0.89, p_{trend}=0.01), but no similar association was evident in those aged <70 years.

Conclusions: Older persons living with a healthy diet and lifestyle were found to be less likely to develop visual impairment over a long-term, regardless of cause. As lifestyle factors are modifiable, these findings, if confirmed, may help to achieve healthy aging and aging productively.

Introduction

Visual impairment is associated with reduced quality of life, increased risk of falls, fractures and all-cause mortality.[375;377;378;400-414] Although visual impairment reflects the severity of ocular disease, the prevalence of visual impairment in a population can reflect the quality of health care and be an indicator of living standards for that particular population. Furthermore, although analysis of ocular diseases is important, the most relevant outcome to patients and medical practitioner's alike is visual impairment.

Many studies and some trials have documented dietary associations with cataract and age-related macular degeneration,[322] the two most common causes of visual impairment. Individual micronutrients, in particular, have been shown to reduce the incidence of both conditions.[20;21;322;415] Equally, overall dietary patterns have shown benefits in the prevention of cataract and other diseases such as cardiovascular disease.[416-420]. However, the associations between overall dietary patterns and visual function in older persons have not been explored.

In this study we investigate the global impact of healthy diet and healthy lifestyle on the 10-year incidence of visual impairment among an older Australian cohort, using the Healthy Eating Index (HEI), developed based on Australian dietary guidelines.

Methods

Study population

We conducted a population-based cohort study of vision, common eye diseases and other health outcomes in an urban, predominantly Caucasian population aged 49 years or older in Blue Mountains, west of Sydney, Australia. At baseline in 1992-4, 3654 participants (82.4%

response) were examined.[211;237] Participants were examined every 5 years; 2335 (75.1% of survivors) at the second examination in 1997-9, and 1952 (76.5% of survivors) at the third in 2002-4. The study adhered to recommendations of the Helsinki Declaration and was approved by the Sydney West Area Human Ethics Committee. Written, informed consent was obtained from all participants.

Visual Impairment

Participants attended a comprehensive medical interview and eye examination by trained technicians. Monocular distance logarithmic of the minimum angle of resolution (LogMAR) visual acuity was measured with an automatically calibrated retro-illuminated chart (85cd/m2) at 8 feet (Vectorvision CSV-100TM, Vectorvision, Inc, Dayton, OH) using forced-choice procedures according to the Early Treatment Diabetic Retinopathy Study (ETDRS) methods. Vision was initially assessed using habitual correction, and proceeded to a 1.2mm pinhole aperture and subjective refraction if fewer than 54 letters were read correctly (6/6 Snellen equivalent).[421] For each eye, visual acuity was recorded as the number of letters read correctly from 0 to 70 (6/60 to 6/3). If no letters on the chart could be identified, visual acuity was assessed as count fingers at 61cm, hand movements, perception of light, or no perception of light.

Visual impairment was defined as visual acuity of less than 6/12 (<39 letters read on the LogMAR chart, allowing for one incorrect letter on the 6/12 line). Visual acuity in this study refers to best corrected acuity, i.e. visual impairment due to eye pathologies which persisted after subjective refraction. Age was defined as the age of participants at the baseline examinations.

An eye was at risk for incident visual impairment if it had a baseline BMES visual acuity of 6/12 or better (≥39 logMAR letters). Incident unilateral visual impairment was defined as those with both eyes at risk at baseline (visual acuity ≥6/12) but who subsequently developed visual impairment in one eye only and the level of incident unilateral visual impairment was defined according to the worse eye. Incident bilateral visual impairment was defined as those with both or one eye at risk but who subsequently developed visual impairment in both eyes. The level of incident bilateral visual impairment was defined according to the better eye. An eye was at risk of incident visual improvement if it had a baseline vision of worse than 6/12 (<39 letters).

Incident visual impairment or improvement at the 5- and 10-year study was determined by comparing participant's visual status at these two follow-up studies with that of the baseline examinations. However, confusion arises when data from the 5- and 10-year examinations are combined statistically, particularly if participants fluctuated between not having and having visual impairment in the three time points. In this analysis, incident visual impairment was defined as persons who developed visual impairment at either follow-up visit as compared to their baseline examination, irrespective of whether their vision improved subsequently in later follow-up examinations. For example, a participant who did not have visual impairment at the baseline study, but developed visual impairment at the 5-year visit from cataract, before having the cataract extracted, such that there was no visual impairment at the 10-year visit, will still be considered as having incident visual impairment in this report. Similarly, a participant with visual impairment at baseline but who improved to not having visual impairment at the 5-year visit due to recent cataract surgery, and then deteriorated again to having visual impairment at the 10-year visit (for example due to macular degeneration) would not be classified as incident visual impairment.

Dietary Assessment

Participants completed a detailed food frequency questionnaire (FFQ). This had 145-items, modified for Australian diet and vernacular from a Willett questionnaire,[214] that incorporated a nine-category frequency scale and standard portion size estimates. This FFQ had reasonable concurrent validity when validated against 4-day weighed food records collected on three occasions in one year (n=79).[214;422]

HEI Development

A modified version of the Healthy Eating Index for Australians (HEIFA), based on the Dietary Guidelines for Adult Australians (DGAA), the Australian Guide to Healthy Eating (AGHE)[423] and the US 2005 Dietary Guidelines Adherence Index (DGAI) were used to establish diet quality. The DGAI takes account of recent changes to the American dietary guidelines and has a focus on energy density,[424] issues also applicable to the Australian population. HEI scores were allocated according to intake of selected food groups and nutrients of each participant and accounted for both variety and quantity of intake. Details of the scoring system can be seen in Appendix 1, with each AGHE guideline having a possible score ranging from 0 to 2. A maximum score of 2 was given to subjects who met the recommendations with proportioned scores for lower intakes. These were then combined to create a final score ranging between 1 and 20.

The HEI was subdivided into two categories, food intake and healthy choice, with scores allocated to reflect intakes from both categories. Food intake components were based on adherence to DGAA recommendations for total intakes of vegetables, fruit, cereals and breads, meat including lean meats, fish, poultry and/or alternatives and dairy as well as low sodium, alcohol, sugar and extra foods intakes. Healthy choice components determined intakes of options with greater dietary benefits including serves of whole grain cereals, lean

red meat, low or reduced fat milk versus whole milk, low saturated fat intake and fish consumption. Cut points for scores were determined from the published recommendations with some exceptions. We replaced the DGAA recommended 2 serves per day of fruit with 3 serves per day and the number of vegetable consumed per day from five serves to seven serves to allow for self reported FFQ overestimation as determined by the validity study.[214]

Non-dietary components of the AGHE were included in the HEI for example the recommendation for preventing weight gain. Half the score related to the ratio of energy intake (EI) to energy expenditure (EE) with maximum score for a ratio between 0.76 and 1.24, defined as the 95% confidence levels of agreement between EI and EE(Black 2000). Levels of physical activity were scored as Metabolic Equivalents (METS) divided into tertiles. Physical activity levels were self reported using questions from the International Physical Activity Questionnaire.[425] Details of walking exercise and performing moderate or vigorous activities were used to calculate METS and subjects in the highest METS tertile scored 1 point which reduced to a 0 point score for subjects in the lowest METs tertile.

Drinking plenty of water and care for food categories from the AGHE was not included in the analysis because data on these items was not available in this study.

Assessment of confounders

The interview included questions about past medical history, including physician-diagnosed history of stroke or myocardial infarction, and lifestyle factors like smoking. Higher educational achievement was defined as attainment of qualifications (certificate, diploma or degree) after leaving school. Participants were asked whether they owned their own home, were renting or living in a relative's home and whether they lived alone, or with a spouse or with other persons. A single measure of systolic and diastolic blood pressure using a

mercury sphygmomanometer was recorded from the first and fifth Korotkoff sounds. Body mass index (BMI) was calculated as weight(kg) /height (m^2). Hypertension was defined as grade 2, if systolic blood pressure >160mmHg and/or diastolic blood pressure of >100mmHg or using anti-hypertensive medications.[225] Diabetes was defined either from past history of diabetes and current diabetes treatment, or from fasting plasma glucose levels ≥7.0mmol/L at examination, using the World Health Organization diabetes classification.[224] Fasting blood samples were processed on the same day for white cell count, haemoglobin, fibrinogen, total cholesterol and high-density lipoprotein (HDL)-cholesterol by the Institute of Clinical Pathology and Medical Research, Westmead Hospital.

Statistical Methods

Statistical analyses were performed using Statistical Analysis Software (version 9, SAS Institute, Cary, NC). We examined the association between baseline HEI score and the 10-year incidence of unilateral visual impairment. Numbers with incident bilateral visual impairment were only 60 which prevented reliable assessment of associations. Participants were divided into quartiles of HEI score. Quartile 1 was used as the reference group.

Person-specific incidence rates were calculated using Kaplan-Meier product-limit survival estimates, to incorporate information from the 5- and 10-year examinations. Cumulative incidence was estimated as (1-Kaplan-Meier estimate) and expressed as a percentage. Discrete linear logistic models were used to assess relationships between dietary variables and incident visual impairment at either of the 2 follow-up time points. The following potential confounders were used: age, gender, qualification level, home ownership, smoking, hypertension, diabetes and history of coronary heart disease. We also adjusted for recent or past visits to an ophthalmologist or optometrist. To examine statistical interaction as a

departure from joint multiplicative effects, cross-product interaction terms were added into the model. Relative risks (RR) and 95% confidence intervals (CI) are presented.

Results

The baseline cohort consisted of 3654 participants. Of these 607 (24.4%) were seen only at the 5-year examinations, 162 (6.5%) were seen only at the 10-year examinations and 1722 (69.1%) were seen at both time points. We thus had 2297 persons without visual impairment at baseline who returned to the 5- and/or 10-year follow-up studies. Those lost to follow-up tended to be younger, to have lower socioeconomic status, and to smoke, but were less likely to have coronary heart disease.

The FFQ was attempted and returned by 3267 baseline participants (89.4%), with 2897 (79.3% of total participants) having sufficiently complete and plausible FFQ data for analysis. Subjects were excluded if over 12 questions were missing, if an entire page remained blank, or if daily energy intakes were <2500 kJ or >18,000 kJ. Participants without usable FFQ data were more likely to be older (mean age 69.3 years vs. 65.3 years), female (15.8% of women vs. 13.5% of men) or current smokers (17.7% vs. 14.2%) than those with usable FFQs.

The study sample consisted of 1963 participants (53.7%) who had reliable dietary assessment and were at risk of development of visual impairment. The cumulative incidence of unilateral, bilateral and second eye visual impairment was 12.4%, 4.6% and 26.5%, respectively. The incidence increased significantly with age (gender-adjusted p-trend≤0.002) with the greatest increase in those aged 65+ years at baseline. Incidence visual impairment was higher in female participants but these differences were only significant in incident

unilateral visual impairment (p=0.04). The most common causes of incident unilateral or bilateral visual impairment over 10 years were cataract (accounting for 54.2% of unilateral visual impairment) and AMD (23.7% of unilateral visual impairment). Glaucoma (3.7%) and other retinal conditions (9.0%) made up most of the remainder.

The mean (SD) HEI in the overall sample was 9.20 (2.33). **Table 6.1** presents the characteristics of the HEI by quartiles of important food groups.

Table 6.2 shows the baseline characteristics of the population by HEI quartiles. Female gender, qualification level, smoking status, HDL-cholesterol level, white cell count and haemoglobin differed across quartiles.

The following two tables use incident unilateral visual impairment as the outcome variable. **Table 6.3** demonstrates the associations between quartiles of HEI and 10-year incident visual impairment. In persons in the highest quartile of HEI compared to the lowest quartile, there was a statistically significant, almost 40% risk reduction of visual impairment in both the age- and sex-adjusted and multivariable-adjusted models (multivariable model, 4th quartile vs. 1st: relative risk, RR, 0.60, 95% confidence interval, CI, 0.40 - 0.89, p_{trend}=0.016). Each unit increase in the HEI was associated with an approximately 10% reduction in the risk of visual impairment (multivariable model: RR, 0.92, 95% CI, 0.87 - 0.98, p_{trend}=0.012). There was no change in effect size after further adjusting for recent or past visits to an ophthalmologist or optometrist.

Table 6.4 shows the associations between quartile of HEI and the 10-year incidence of visual impairment in two stratified age groups (less than or greater than 70 years). The cross-product interaction term for age and HEI was significant, p=0.03. In persons younger than 70

years of age, there was the suggestion of a reduction in risk of visual impairment comparing the highest to the lowest quartile of HEI, although this result and the trend were not significant (multivariable model, 4th quartile vs. 1st: RR, 0.75, 95% CI, 0.43 – 1.29, p_{trend}=0.41). There was a significant reduction in the risk of visual impairment among persons 70 years or greater who had the highest HEI scores (multivariable model, 4th quartile vs. 1st: RR, 0.49, 95% CI, 0.27 – 0.89, p_{trend}=0.01).

Stratification by gender (data not shown) demonstrated no relationship between HEI and visual impairment in men (multivariable model, 4th quartile vs. 1st: RR, 0.59, 95% CI, 0.28 – 1.26, p_{trend}=0.32) but a significant reduction in risk in women (multivariable model, 4th quartile vs. 1st: RR, 0.57, 95% CI, 0.35 – 0.93, p_{trend}=0.03). However, the interaction term for gender and HEI was not significant, p = 0.30.

We analysed our data using bilateral visual impairment (visual impairment in better eye at 10 years follow-up) as the outcome, but there were fewer cases for this variable (data not shown). The effect magnitudes and direction were similar but did not reach significance.

Discussion

Visual impairment is an important factor affecting independent living of individuals and their quality of life.[375-378;400-414] There has been no previous study on the relationship of overall visual impairment and lifestyle factors. In this study, we have shown that, in our entire cohort, individuals who consume a diet that closely approximates dietary guidelines and undertake physical activity (as reflected by a higher HEI) are less likely to develop visual impairment in the long-term. There was a significant age interaction, with persons 70

years or older with a higher HEI score, more likely to have risk reduction in visual impairment than persons younger than 70 years.

Two studies have examined associations with visual impairment in a slightly different context. In a Spanish study of visually impaired or blind children, researchers found the patterns of dietary consumption were the same as that observed in non-visually impaired children in quantity and quality, but children with visual impairment were more likely to be overweight or obese.[426] This finding may be explained by the fact that visually impaired children are less likely to participate in outdoor physical activities compared to their peers. In a Finnish prospective study, an increased incidence of liver and lung cancer was found in blind persons, which the researchers suggested was strongly related to lifestyle and socioeconomic factors.[427]

Our study suggests that regardless of the cause of visual impairment, poor lifestyle and dietary habits may lead to any chronic, ageing-related eye disease and cause significant visual impairment. Furthermore, the significant association observed in older persons may suggest that the longer period of poor dietary behaviour, the more likely the development of visual impairment. It is possible that in the younger age group (<70 years of age) the number with incident visual impairment was too few to have sufficient study power to detect a significant association. Even though many nutritional studies have investigated the associations between eye diseases and dietary factors,[20;21;322;415] our finding is of importance because it implies that lifestyle factors over an individual's lifetime play a significant role in the occurrence of visual impairment. Our findings also suggest an explanation for the relationship between visual impairment and mortality which has been documented in many studies.[376-378;411-414;428]

There can be many alternative explanations for our findings. Visual impairment is reflective of ocular diseases, but can indicate socioeconomic factors or access to ophthalmic health services. In our sample, HEI index scores were lower in those without post-school qualifications or ownership of their home. Previous studies have shown that higher socioeconomic status was related to the increased use of ophthalmology services.[429] Thus persons with a low HEI could have fewer visits to an ophthalmologist, which might explain our findings. However adjustment for the number of visit to ophthalmology services in the past did not change our findings of the associations. Visually impaired persons may also be less likely to have a healthy diet due to difficulty in food purchase or preparation. However, our study was prospective and dietary factors were collected at baseline, well before the development of incident unilateral visual impairment over the follow-up period, and thus this "reverse association" is unlikely to be an explanation for our findings. Indication bias, where diets were changed in relation to systemic diseases, is also less likely to be an explanation for our findings due to the prospective nature of our study.

Strengths of our study include its prospective nature with long-term follow-up of a well-defined and stable population-based sample. Detailed data collection allowed assessment of a wide range of confounders. Our food frequency questionnaire was validated and comprehensive in that it provided data about wholegrain cereals, vegetables, low-fat milk products that were able to be used in the calculation of the HEI.

Our study has several limitations. There were a relatively high proportion of participants with missing FFQ data, who were likely to be older and current smokers, which may have biased our findings in either direction. Our food frequency questionnaire likely overestimated fruit and vegetable intakes, and underestimated overall energy intake.[214] However we attempted to account for this in our calculation of the HEI score, by replacing

the number of serves of fruits and vegetables as recommended by the guidelines with a higher number of serves. We also attempted to address potential confounders such as socioeconomic status and smoking in our analysis. Another limitation of our study is the use of a single blood pressure measurement, which may not accurately reflect a participant's 'usual' blood pressure over time.

In summary, we have shown that persons with a high HEI score, which represents beneficial dietary and physical activity factors, was associated with a lower risk of incident visual impairment in either eye over 10 years. Although there may be many potential explanations for this finding, and a need for confirmation of this finding in other populations, it is a potentially significant finding given that it highlights the importance of lifestyle factors, beyond smoking, in ageing-related eye disease.

Table 6.1. Description of the range of Healthy Eating Index scores in each quartile, and their relationship to selected food groups – lean red meat, fish and fruit and vegetable consumption (mean, 95% confidence interval)

	Mean	Min	Max	Leaner red meat (grams)	Fish (grams)	Fruit (grams)	Vegetables (grams)
Q 1	6.23	2.55	7.47	41.50 (37.22, 45.79)	17.30 (15.77, 18.91)	259.07 (238.55, 279.59)	395.01 (376.91, 413.12)
Q 2	8.37	7.50	9.20	53.96 (49.76, 58.16)	25.68 (25.34, 28.02)	326.55 (306.54, 345.78)	424.25 (408.28, 441.23)
Q 3	10.00	9.20	10.83	58.73 (54.86, 62.60)	29.63 (27.35, 31.92)	373.47 (351.69, 395.26)	454.25 (438.98, 469.52)
Q 4	12.20	10.85	16.30	59.03 (55.10, 62.96)	36.95 (34.08, 39.82)	456.83 (430.71, 482.95)	505.37 (488.23, 522.51)
P for trend				<0.0001	<0.0001	<0.0001	<0.0001

Table 6.2. Baseline characteristics of the population by quartiles of Healthy Eating Index

Baseline characteristics	Quartile 1 (n=489)	Quartile 2 (n=490)	Quartile 3 (n=492)	Quartile 4 (n=492)	P for trend
Age	63.1	63.6	63.0	64.1	0.17
Female (%)	46.2	48.6	61.4	72.2	<0.0001
Post secondary school qualification (%)	57.4	61.0	65.1	63.8	0.02
Current smoker (%)	19.6	14.6	9.9	6.5	<0.0001
Body-mass index	26.4	26.8	26.5	26.0	0.068
Systolic blood pressure	145	144	145	145	0.32
Diastolic blood pressure	84	83	83	83	0.19
Hypertension (%)	43.0	37.2	41.8	44.6	0.35
Diabetes (%)	4.7	6.5	6.9	6.5	0.23
History of coronary heart disease (%)	13.3	11.0	14.9	14.7	0.25
History of stroke (%)	2.9	2.9	2.7	3.5	0.64
Fasting serum cholesterol (mmol/L)	6.0	6.1	6.0	6.1	0.47
Fasting serum HDL cholesterol (mmol/L)	1.4	1.4	1.4	1.5	0.02
Fasting glucose (mmol/L)	5.2	5.3	5.1	5.1	0.09
White cell count ($x10^9$)	6.5	6.6	6.4	6.1	<0.0001
Haemoglobin (g/dL)	150.1	150.0	149.0	146.5	<0.0001
Fibrinogen (mg/dL)	4.0	4.0	4.0	4.1	0.32

Table 6.3. Association between Healthy Eating Index (HEI) and 10-year incidence of non-correctable visual impairment (in at least one eye)

HEI		Incident visual impairment (<6/12)		
Quartile	Median (Range)	N cases / N at risk	Age-sex adjusted Relative risk (95% CI)	Multivariable* adjusted Relative risk (95% CI)
1st (lowest)	6.47 (2.55 – 7.47)	74 / 489	1 (referent)	1 (referent)
2nd	8.40 (7.50 – 9.19)	68 / 490	0.74 (0.51, 1.07)	0.69 (0.46, 1.02)
3rd	10.00 (9.20 – 10.83)	61 / 492	0.70 (0.48, 1.01)	0.68 (0.46, 1.02)
4th (highest)	11.95 (10.85 – 16.30)	60 / 492	0.59 (0.41, 0.86)	0.60 (0.40, 0.89)
P for trend			0.007	0.016
Continuous (per 1unit increase)			0.92 (0.87, 0.98)	0.92 (0.87, 0.98)
P value			0.007	0.012

* adjusted for age, gender, qualification, home ownership, smoking, hypertension, diabetes and history of coronary heart disease

Table 6.4. Association between Healthy Eating Index (HEI) and 10-year incidence of non-correctable visual impairment (in at least one eye), stratified by age

HEI		Incident visual impairment (<6/12)		
Quartile	Median (Range)	N cases / N at risk	Age-sex adjusted Relative risk (95% CI)	Multivariable* adjusted Relative risk (95% CI)
		Age < 70		
1st (lowest)	6.43 (2.77, 7.47)	33 / 376	1 (referent)	1 (referent)
2nd	8.40 (7.50, 9.20)	28 / 374	0.74 (0.44, 1.26)	0.64 (0.36, 1.13)
3rd	10.00 (9.20, 10.83)	31 / 385	0.84 (0.50, 1.41)	0.78 (0.45, 1.34)
4th (highest)	11.90 (10.85, 16.30)	33 / 364	0.79 (0.48, 1.33)	0.75 (0.43, 1.29)
P for trend			0.48	0.41
		Age ≥ 70		
1st (lowest)	6.63 (2.55, 7.47)	41 / 113	1 (referent)	1 (referent)
2nd	8.43 (7.50, 9.15)	40 / 116	0.76 (0.45, 1.29)	0.81 (0.46, 1.41)
3rd	9.90 (9.20, 10.80)	30 / 107	0.59 (0.33, 1.01)	0.64 (0.35, 1.16)
4th (highest)	12.04 (10.85, 15.20)	27 / 128	0.44 (0.25, 0.77)	0.49 (0.27, 0.89)
P for trend			0.002	0.01
P for interaction = 0.03†				

* adjusted for age, gender, qualification, home ownership, smoking, hypertension, diabetes and history of coronary heart disease

†cross-product multiplicative term: age by HEI

Part 7

Conclusion

Chapter 13: Implications of findings from this thesis

Diet is a readily modifiable lifestyle behaviour affecting an individual's health on a daily basis over an entire lifetime. The common public perception of 'we are what we eat' precisely illustrates its importance. Research on age-related diseases and diet demonstrates that our ability to withstand the onset of ageing-related eye and systemic diseases is assisted by having a healthy diet and lifestyle in early childhood or as a young adult.[1] There is consensus about the importance of dietary patterns in age-related diseases such as cardiovascular disease,[316;415;420;430;431] but in the area of dietary factors and age-related eye disease, opinions are mixed.[19;20;82;432] In this PhD research project, I have examined some newer and some novel dietary areas in relation to age-related eye diseases in a population-based, homogenous, older Australian cohort that has been meticulously studied over 15 years.

The difficulties in nutritional research are that dietary factors and nutrients are consumed in combination and therefore their effect is complex and inter-related, in addition to being affected by various lifestyle factors. This can be appreciated by the observation that serum levels of a nutrient do not necessarily correlate well with dietary intakes, as they may be affected by intestinal absorptive capacity, overall health of the individual, ageing, decreased consumption of foods and interactions with many medications.[415;433] The isolation of specific nutrients for study may not reflect the actual influence of these nutrients on the body. However, study of a single nutrient may be advantageous if this single nutrient is more prevalent in one part of the body or the nutrient has specific physiological actions. The nutrients, lutein and zeaxanthin, have particular high concentrations in the macula lutea of the retina and specific roles in maintaining macular pigment density, therefore it may be reasonable to study the intake of these nutrients in isolation.[70] For most food items and nutrients, analysis using overall dietary patterns or intake scores that include other lifestyle factors seems judicious given the interdependency of many

nutrients, such as vitamin C and E,[434-436] and the evidence of the benefits of certain dietary patterns such as the Mediterranean diet against cardiovascular mortality.[417-419] Whole foods, with their multiple nutrients, have also shown to be more frequently efficacious, in comparison with supplements. In this thesis, I have attempted to study whole foods, rather than just the isolated nutrients, and in Chapter 12, I have utilised a dietary pattern index in relation to overall visual impairment that encompasses a few important age-related eye diseases.

Retinal microvasculature analysis is an exciting new field with research to date demonstrating a relationship between a spectrum of clinical and sub-clinical cardiovascular, cerebrovascular and systemic diseases.[4-17] In Part 3, this thesis presents a new statistical method of using the retinal vessel calibre as an independent variable in analysis, as well as examining, for the first time, the associations of dietary factors with retinal vessel calibre, in predicting longitudinal vascular mortality. The statistical models described account for the correlation between an individual's retinal arteriolar size and retinal venular size (Chapter 3), which corresponds with the biologically-based theory that individuals who have larger-sized arteries, will tend to have larger-sized veins. This method, used in analyses, examines each vessel type independent of the other by accounting for the shared variance between veins and arteries, and thus provides more precise estimates of the associations with each vessel type.

This thesis is the first to investigate the longitudinal associations of dietary factors with retinal vessel calibre, and to document that the beneficial effects of healthy diet on lower risk of stroke is partly explained by the effect of healthy diet on the small vessels (Chapter 5, 6). Dietary factors, such as fish consumption, fibre and glycemic index, affect the retinal microvasculature, and these microvasculature changes may be an intermediary pathway to systemic vascular

disease. This finding has clinical implications for retinal microvascular analysis in the treatment of vascular disease. Retinal fundus photographs may be used as a sub-clinical endpoint to assess treatment effects of dietary intervention. The mechanisms through which dietary changes are thought to ameliorate leading to vascular benefits deserve further investigation.

A novel contribution of this thesis, is in the study of a well-known, but poorly studied retinal microvascular sign, the enhanced retinal arteriolar reflex. This sign is widely recognised as a sign of hypertensive retinopathy by ophthalmologists and other medical specialities and has been for almost 200 years.[8;257;262-266] However, its relationship to hypertension, cardio- and cerebrovascular disease outcomes has only been documented in case series.[257;262-266] In a general older population sample, I documented that age was strongly inversely associated with the mild level of this sign, and blood pressure was only associated with the marked level of this sign. These findings suggest that caution should be used in employing this sign to define presence of hypertensive changes and alternative explanations for the presence of this sign should be pursued.

In this direction, Chapter 7 provides further characterisation of this sign, demonstrating that this enhanced retinal arteriolar light reflex is more prevalent in those persons who have a higher intake of antioxidants. This raises the intriguing idea that the enhanced retinal arteriolar light reflex may be related to healthful dietary factors and behaviours.

Dietary interventions have been definitively shown to reduce progression of age-related macular degeneration (AMD).[19-21] Part 4 demonstrates two new and significant findings that may potentially result in crucial dietary interventions. Firstly, persons with a diet low in mean glycemic index have a reduced risk of early AMD. This finding is important because it specifies

a dietary practice that might lead to the primary prevention of AMD. Another finding from this thesis is that egg consumption may prevent late AMD. This outcome is novel in the sense that eggs as a whole food were thought to have harmful health effects, resulting mainly from a concern regarding the possible raising of serum cholesterol levels. More recently, however, there has been a reversal of this perception of eggs, with a documentation of their myriad health benefits.[341] Lately, eggs have been shown to be a highly bioavailable source of lutein and zeaxanthin,[347] which increases their relevance to AMD and the importance of these findings.

Protein and riboflavin consumption has been consistently associated with lower risk of cataract, a principal cause of visual impairment, more burdensome in developing countries due to diminished access to cataract surgical services. This thesis has demonstrated a new finding, that legume consumption (even in those consuming meats) was protective for incidence of cataract, and has confirmed protein and riboflavin relationships. Fats and cataract incidence have only been studied by one group.[163-165] Omega-3 fatty acids, fish and nuts are healthful aspects of diet that resulted in a reduction in incidence of cataract in our cohort. These findings are important for developing countries in particular, where dietary interventions may reduce the burden of illness for health services and individuals. Moreover, since nuclear cataract is an ageing biomarker, our results suggest that these foods may be advantageous in delaying the overall ageing process, an idea that deserves further study.

Finally, in a novel way of looking at an individual's lifestyle and the development of functional vision problems as they get older, this thesis has demonstrated a clinically, (and easily understood by the populace) result of good diet and physical activity in preventing long-term visual impairment. This finding underscores the importance of lifestyle factors to all forms of

eye disease, akin to the relationship between lifestyle factors and other systemic diseases such as cardiovascular disease.

There are inevitable limitations in this project on dietary associations. In particular, dietary intake was self-reported, using a validated food frequency questionnaire, which generates an approximation of quantities of intake. The population studied was a homogenous, Caucasian population that limits generalisation of the study findings to other ethnicities. Moreover, as aforementioned, dietary patterns or whole foods may be more relevant than individual nutrients, though this thesis has examined both. Finally, all dietary observational studies are affected by possible incomplete control of healthy lifestyle variables that may confound the analysis. Overall, the study has a number of strengths. Its population-based, long-term follow-up cohort study design, standardised examination protocol and uniform ascertainment of associated characteristics permits reliable generalisation of findings to the population from which the samples were drawn.

This study has begun to set some future directions for research. Firstly, dietary factors in relation to retinal microvascular signs need confirmation in other populations, especially in relation to systemic diseases such as stroke. If confirmed, this may lead to clinical trials using fundus photographs to assess impact of dietary interventions. The AREDS II trial[83] is expected to provide information regarding lutein, zeaxanathin and omega-3 fatty acid supplements for delaying the progression to late AMD. However, these nutrients need assessment in the context of primary prevention of AMD and in the context of overall dietary patterns. Interventional trials are recommended, especially in relation to these two nutrients, in large scale populations

for the prevention of or to retard the progression of cataract. These trials, in relation to cataract, may be also useful in developing countries.

Finally, as has been amply demonstrated in the retinal microvasculature, pathological changes and ageing-related changes are similar. As stated by Najjer et al,[249] perhaps age in not an immutable risk factor, and may be amenable to dietary intervention.

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Appendix

Appendix: Modified version of Healthy Index for Australians (HEIFA), based on Australian Dietary Guidelines and the Australian Guide to Healthy Eating, for use with the Blue Mountains Eye Study.

Guideline	Food Intake	Healthy choice	Maximum Score
Eat plenty of vegetables, legumes and fruit	Total serve vegetables per day* 7 serves = 0.5 5.6 serves =0.4 4.2 serves = 0.3 2.8 serves = 0.2 1.4 serve = 0.1 Serves fruit per day** 3 serves = 1 2 serve = 0.5	Variety score If ≥ 1 serves/day green = 0.1 If ≥ 1 serves/day orange = 0.1 If ≥ 1 serves/day of cruciferous = 0.1 If ≥ 1 serves/day of tuber or bulb = 0.1 If ≥ 0.5 serves/day of legumes = 0.1.	2
Eat plenty of cereals, preferably wholegrain	Serves cereals /day Women 4 serves=1 3 serves = 0.75 2 serves = 0.5 1 serve = 0.25 Men 6 serves = 1 5 serves = 0.83 4 serves = 0.66 3 serves = 0.5 2 serves = 0.33 1 serve = 0.166	Wholegrain / meal cereal serves Women 4 serves=1 3 serves = 0.75 2 serves = 0.5 1 serve = 0.25 Men 6 serves = 1 5 serves = 0.83 4 serves = 0.66 3 serves = 0.5 2 serves = 0.33 1 serve = 0.166	2
Include lean meats, fish, poultry and/ or alternatives	≥1 serve meat/alternative/day = 1.5	≥3 serves lean red meat / week = 0.5 (i.e. > 0.428 /day)	2
Include milk, yoghurts, cheese and/or alternatives	≥2-3 serves/ day = 1.5 >3-4 serves/day=1.0 1-<2 serve daily = 1.0 >4 serves daily = 0.5 0-<1 serves daily = 0	If quantity of skim/ low fat > whole milk = 0.5 If quantity of skim / low fat=whole milk=0.25 If whole milk > skim milk/ low fat milk = 0	2

Guideline	Food Intake	Healthy choice	Maximum Score
Drink plenty of water	Not scored		
Limit saturated fat and moderate total fat intake		Sat fat < 10% energy = 1 Sat fat 10-12% energy = 0.5 Sat fat >12% energy = 0 Fish ≥ 2 serves / week = 1 Fish 1-<2 serves/week = 0.5 Fish <1 serve/week = 0	2
Choose foods low in salt		≤ 40 mmol (920mg) sodium /day = 2 40- 100 mmol (920-2300mg) sodium/day = 1 >100mmol/day (2300mg) = 0	2
Limit alcohol intake if you choose to drink		Women 0g alcohol = 0 >0 – <10g = 2 10 - <20 g = 1 >20g = 0 Men 0g alcohol = 0 >0-<10g = 1 10-<20g = 2 >20g = 0	2
Consume only moderate amounts of sugars and foods with added sugars	Sugar <15% total energy = 2 Sugar 15-<20% total energy = 1 Sugar≥20% energy = 0		2
Extra foods, not essential to provide nutrients and may be high in salt, fat or sugar***	Women <2.5 serves/day = 2 2.5 - 4 serves = 1 >4 serves = 0 Men < 3 serves/day = 2 3-5 serves = 1 >5 serves = 0		2
Prevent weight gain: be		Energy intake / energy expenditure	2

Guideline	Food Intake	Healthy choice	Maximum Score
physically active and eat according to energy needs		0.76 – 1. 24 = 1 <0.76 = 0 >1.24 = 0 Physical activity METs Lowest tertile = 0 Middle tertile = 0.5 Highest tertile = 1	
Care for food	Not scored		
Total score			**20**

**Vegetables: 7 serves, as indicated by weighed food records (FFQ over-estimates) (replacing 5 serves)*

***Fruit: 3 serves, as indicated by weighed food records (FFQ over-estimates) (replacing 2 serves)*

**** Extra foods: 2.5 serves for women and 3 serves for men, as indicated by weighed food records (FFQ over-estimates)*