High Glycemic Index Diets Accelerate Retinal Aging and Increase Cellular Stress

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A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Biochemical and Molecular Nutrition

Tufts University

Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy

2011

i. Abstract

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly. Pharmacological treatments are not available for the majority of AMD patients, emphasizing the need for preventative care. Unfortunately, intervention trials have not yet identified any particular nutrient that can prevent or delay the onset of the early stages of disease. However, epidemiological analyses of several cohorts have shown that people who consume low glycemic index (GI) diets have a reduced risk for the onset of early AMD, as well as progression through the stages of AMD, relative to those people consuming high GI diets. The glycemic index (GI) measures the rise in blood glucose induced by a particular carbohydrate, relative to the rise in blood glucose evoked by carbohydrate from a standard food such as white bread. Despite the consistency of the observation between GI and AMD risk, there is no biological data to confirm this association or explain its mechanism.

Using a dietary hydroquinone (HQ) model to induce early AMD in 17- and 23.5-month-old C57BL/6 mice, we found that in mice fed either a high or low GI diet, AMD-like lesions were more prominent in the older mice. Within each age group, AMD-like lesions were also more prominent in the higher GI-fed mice. These effects appeared to be independent of the presence of HQ, suggesting that the high GI-fed C57BL/6 mouse can be used as a model for early AMD. In addition to these lesions, mice fed the higher GI diet also accumulated advanced glycation end-products (AGEs) in their retinas.

AGEs are by-products of glucose metabolism that have been associated with a variety of chronic diseases such as AMD, diabetes, kidney disease, and cardiovascular disease. We observed systemic accumulation of AGEs and protein carbonyls (another marker of oxidative stress) in a variety of tissues from mice fed the higher GI diet. Our data suggest that AGE and carbonyl accumulation in these tissues may be due to insufficient proteolytic capacity. Tissues of higher GI-fed mice that showed greater accumulation of these modified proteins also showed relatively lower levels of proteasome activity. Confirming the systemic nature of the high GI stress, the accumulation of AGEs in tissues of higher GI-fed mice was also associated with higher fasting glucose and insulin levels, impaired glucose tolerance, and higher levels of glycated hemoglobin.

Our data confirm human epidemiologic data and show that consumption of a higher GI diet increased the appearance of AMD-like lesions in mice. The higher GI diet impaired glucose clearance, increased levels of glycated hemoglobin, and was associated with systemic accumulation of AGEs in tissue. Our model suggests that GI modulates AMD risk through the accumulation of AGEs in the retina. Glycative-stress-induced changes in activity of the ubiquitin proteasome system may be one mechanistic link between dietary GI, AGE accumulation and tissue damage such as AMD-like lesions. This link between dietary GI and disease is not only relevant to AMD, but may also apply to other chronic diseases which are associated with both high GI diets and AGE accumulation.

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Chapter 1. Introduction

1.1 General Statement of the Problem and its Significance

Age-related macular degeneration (AMD) afflicts nearly 7.2 million Americans, and current therapies to alleviate its ensuing vision loss are effective in less than 10% of these patients (1-9). Numerous pharmacological treatments including antioxidants, anti-inflammatory and neurotrophic agents, and visual cycle modifiers are in the research pipeline, but few have yet successfully passed clinical testing to aid the remaining 90% of AMD patients (10, 11). Such a lack of effective treatment is daunting, considering the estimation that over 15 million Americans will have AMD by the year 2050 (12, 13).

Vision loss that accompanies AMD takes a significant toll on an individual's physical, mental, and emotional well-being. The abilities to read, write, and recognize faces are significantly reduced in AMD patients, along with the capacity to perform simple daily tasks. Without clear vision, mobility is impaired, and consequently, AMD patients often experience a greater number of falls than those with preserved vision (14-20). Furthermore, while many of these individuals may need and qualify for low-vision rehabilitative services, most do not have access to such services (21-23). Not surprisingly, these physical limitations contribute to mental stress and anxiety, leading to more symptoms of depression among AMD patients than in elderly without vision loss (24-26).

Several different assessment tools have been developed to characterize the social and emotional impact of vision loss. Two such tools are the Quality of Well-Being Scale and the Profile of Mood States, which indicate that AMD

patients exhibit emotional distress similar to that experienced by those who suffer from chronic diseases such as arthritis, chronic obstructive pulmonary disease, and acquired immune deficiency syndrome (25). One of the strongest contributors to this emotional distress is the fear of future vision loss. Greater emotional stress was observed among patients with legal blindness in only one eye compared to the emotional stress experienced in those legally blind in both eyes (25, 27). Another assessment of quality of life is the Time Trade-off Utility Value. This value reflects the years of life that a patient is willing to give up, if only they are cured of their current debility (28). Utility values reported by patients with mild AMD are comparable to those reported by cancer patients and those with HIV infections. Patients with moderate forms of AMD report values similar to patients with tuberculosis and hip fractures, and advanced AMD patients report values that reflect the quality of life experienced by patients with advanced prostate cancer and bedridden stroke patients (29).

The physical, emotional, and mental burden of AMD on the individual is tremendous, and unfortunately this burden also extends to the greater community through strain on financial resources. AMD impacts the public health system though direct costs for treatment as well as indirect costs such as the need for at-home healthcare and lost productivity in the work force (30). Direct costs for AMD therapy in the US are estimated to be approximately 575 million dollars per year, a number which is only expected to increase if effective new therapies are developed (31). Although the prevalence of AMD varies between countries, multinational studies have shown that the drain of this condition on the

public health care system is not unique to the US. For both direct and indirect costs associated with bilateral neovascular AMD, these patients use approximately 5 times the national healthcare resources as age-matched controls with minimal vision loss. In countries such as Germany and Canada, the costs are even greater (32). In addition to these expenses, AMD patients with visual impairment are also less productive workers, and contribute less to the national economy, as reflected in the national gross domestic product (GDP). Visual impairment often results in a salary reduction, compared to those with healthy vision, and a substantial number of jobs are lost once AMD progresses into the advanced stages. Although many advanced AMD patients are elderly, it is estimated that approximately 10% of people over the age of 65 are still in the work force. Visual disturbances caused by neovascular AMD or geographic atrophy significantly impair the ability of the elderly (≥65 yrs) to work, resulting in a drop of the US GDP of about 8 billion dollars (29).

This devastating impact of AMD on individual and community well-being has led to the investigation of preventative therapies such as vitamin supplementation, in addition to exploration of pharmaceutical treatments to delay the onset of AMD. It has been estimated that the combination of vitamin supplementation with anti-inflammatory and photodynamic therapy may help reduce AMD-related visual impairment by 34%, relative to a reduction of only 16% when vitamin supplementation is excluded from this combination (13). Many observational studies have been conducted to find which particular vitamins confer the most retinal benefit (33-60). Thus far, the most effective

combination of micronutrients tested in an intervention study is a cocktail of vitamins C, E, beta-carotene, zinc and copper. However, this mixture only helped to reduce the risk of AMD progression in those who have already developed the disease. This supplement did not prevent the onset of disease. A clinical trial is underway to investigate the roles of lutein, zeaxanthin and omega-3 fatty acids in slowing down AMD progression, but this trial will not test the ability of these nutrients to delay AMD onset (11). At the moment there is still a great need for therapies that will prevent or delay early AMD. The impact of these therapies will be dramatic. It estimated that reduction of risk for late-stage AMD in those with high-risk eyes would halve the rate of AMD-related blindness (61). In addition to preserving vision, these therapies would not only preserve quality of life for millions of elderly, but will substantially alleviate public health costs (62).

1.2 Statement of Hypothesis

Epidemiological evidence suggests that risk for the onset of AMD is increased in those who consume high glycemic index (GI) diets (63, 64). The GI is a relative measure of the rise in blood glucose triggered by carbohydrates in a particular food, compared to the rise in blood glucose evoked by carbohydrates of a standard food such as glucose or white bread (65). Consumption of high GI diets is associated with poor glucose control in diabetics, as well as high serum levels of advanced glycation end products (AGEs) (66, 67). AGEs are proteins that have been non-enzymatically modified by glucose and can be cytotoxic in

cells (66-76). Accumulation of AGEs is associated with a number of chronic diseases, including AMD (77-91). Abnormal protein accumulation, such as that of AGEs, is usually managed by cellular protein quality control pathways such as the ubiquitin proteasome system (UPS) (92). It is possible that AGEs may accumulate due to a limited availability of glycated proteins for degradation or because proteolytic capacity of the UPS is insufficient under glycative stress (93, 94).

We will test the hypothesis that mice fed a low GI diet will have a limited accumulation of AGEs and develop fewer AMD-like lesions compared to mice fed a high GI diet. We predict that preservation of an efficient UPS in the low GI-fed mice will help minimize AGE accumulation and therefore preserve retinal health. Elucidation of the mechanism underlying the relationship between GI and AMD will not only shed light on AMD pathogenesis, but will clarify the role of dietary GI in disease prevention.

1.3 References

1. Bressler NM. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2. Arch Ophthalmol. 2001 Feb;119:198-207.

2. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-

related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology. 2009 Jan;116:57-65 e5.

3. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006 Oct 5;355:1419-31.

4. Gragoudas ES, Adamis AP, Cunningham ET, Jr., Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med. 2004 Dec 30;351:2805-16.

5. Friberg TR, Tolentino M, Weber P, Patel S, Campbell S, Goldbaum M. Pegaptanib sodium as maintenance therapy in neovascular age-related macular degeneration: the LEVEL study. Br J Ophthalmol. 2010 Dec;94:1611-7.

6. Forte R, Cennamo G, Finelli M, Cesarano I, D'Amico G, De Crecchio G, Cennamo G. Intravitreal triamcinolone, bevacizumab and pegaptanib for occult choroidal neovascularization. Acta Ophthalmol. 2010 Dec;88:e305-10.

7. Tufail A, Patel PJ, Egan C, Hykin P, da Cruz L, Gregor Z, Dowler J, Majid MA, Bailey C, et al. Bevacizumab for neovascular age related macular degeneration (ABC Trial): multicentre randomised double masked study. Bmj. 2010;340:c2459.

8. Biarnes M, Mones J, Alonso J, Arias L. Update on Geographic Atrophy in Age-Related Macular Degeneration. Optom Vis Sci. 2011 Apr 28.

9. Klein R, Chou CF, Klein BE, Zhang X, Meuer SM, Saaddine JB. Prevalence of age-related macular degeneration in the US population. Arch Ophthalmol. 2011 Jan;129:75-80.

10. Meleth AD, Wong WT, Chew EY. Treatment for atrophic macular degeneration. Curr Opin Ophthalmol. 2011 May;22:190-3.

11. Zarbin MA, Rosenfeld PJ. Pathway-based therapies for age-related macular degeneration: an integrated survey of emerging treatment alternatives. Retina. 2010 Oct;30:1350-67.

12. Friedman DS, O'Colmain BJ, Munoz B, Tomany SC, McCarty C, de Jong PT, Nemesure B, Mitchell P, Kempen J, Group. EDPR. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol. 2004;122:564-72.

13. Rein DB, Wittenborn JS, Zhang X, Honeycutt AA, Lesesne SB, Saaddine J. Forecasting age-related macular degeneration through the year 2050: the potential impact of new treatments. Arch Ophthalmol. 2009 Apr;127:533-40.

14. Ivers RQ, Norton R, Cumming RG, Butler M, Campbell AJ. Visual impairment and risk of hip fracture. Am J Epidemiol. 2000 Oct 1;152:633-9.

15. Desrosiers J, Wanet-Defalque MC, Temisjian K, Gresset J, Dubois MF, Renaud J, Vincent C, Rousseau J, Carignan M, Overbury O. Participation in daily activities and social roles of older adults with visual impairment. Disabil Rehabil. 2009;31:1227-34.

16. Dargent-Molina P, Favier F, Grandjean H, Baudoin C, Schott AM, Hausherr E, Meunier PJ, Breart G. Fall-related factors and risk of hip fracture: the EPIDOS prospective study. Lancet. 1996 Jul 20;348:145-9.

17. Wood J, Lacherez P, Black A, Cole M, Boon M, Kerr G. Risk of falls, injurious falls, and other injuries resulting from visual impairment among older

adults with Age-related Macular Degeneration. Invest Ophthalmol Vis Sci. 2011 Apr 7.

18. Finger RP, Fenwick E, Marella M, Dirani M, Holz FG, Chiang PP, Lamoureux EL. The Impact of Vision Impairment on Vision-Specific Quality of Life in Germany. Invest Ophthalmol Vis Sci. 2011 Feb 25.

19. Klein R, Klein BE, Tomany SC, Moss SE. Ten-year incidence of age-related maculopathy and smoking and drinking: the Beaver Dam Eye Study. Am J Epidemiol. 2002;156:589-98.

20. Hassell JB, Lamoureux EL, Keeffe JE. Impact of age related macular degeneration on quality of life. Br J Ophthalmol. 2006 May;90:593-6.

21. Culham L, Ryan B, Jackson A. Low vision services for vision rehabilitation in the United Kingdom. Br J Ophthalmol. 2002;86:743-7.

22. Lovie-Kitchin J, Keefe J, Taylor H. Referral to low vision services by optometrists. Clin Exp Optom. 1996;79:227-34.

23. Stelmack J. Quality of life of low vision patients and outcomes of low vision rehabilitation. Optom Vis Sci. 2001;78:335-42.

24. Rovner BW, Casten RJ. Activity loss and depression in age-related macular degeneration. Am J Geriatr Psychiatry. 2002 May-Jun;10:305-10.

25. Slakter JS, Stur M. Quality of life in patients with age-related macular degeneration: impact of the condition and benefits of treatment. Surv Ophthalmol. 2005 May-Jun;50:263-73.

26. Soubrane G, Cruess A, Lotery A, Pauleikhoff D, Mones J, Xu X, Zlateva G, Buggage R, Conlon J, Goss TF. Burden and health care resource utilization in

neovascular age-related macular degeneration: findings of a multicountry study. Arch Ophthalmol. 2007 Sep;125:1249-54.

27. Williams RA, Brody BL, Thomas RG, Kaplan RM, Brown SI. The psychosocial impact of macular degeneration. Arch Ophthalmol. 1998 Apr;116:514-20.

28. Froberg D, Kane R. Methodology for measuring healthstate preferences. II. Scaling methods. Journal of Clinical Epidemiology. 1989;42:459-71.

29. Brown MM, Brown GC, Stein JD, Roth Z, Campanella J, Beauchamp GR. Age-related macular degeneration: economic burden and value-based medicine analysis. Can J Ophthalmol. 2005 Jun;40:277-87.

30. Frick KD, Gower EW, Kempen JH, Wolff JL. Economic impact of visual impairment and blindness in the United States. Arch Ophthalmol. 2007 Apr;125:544-50.

31. Rein DB, Zhang P, Wirth KE, Lee PP, Hoerger TJ, McCall N, Klein R, Tielsch JM, Vijan S, Saaddine J. The economic burden of major adult visual disorders in the United States. Arch Ophthalmol. 2006 Dec;124:1754-60.

32. Cruess AF, Zlateva G, Xu X, Soubrane G, Pauleikhoff D, Lotery A, Mones J, Buggage R, Schaefer C, et al. Economic burden of bilateral neovascular age-related macular degeneration: multi-country observational study. Pharmacoeconomics. 2008;26:57-73.

33. Weikel K, Taylor, A. Nutritional Modulation of Cataract and Age-Related Macular Degeneration. Duanes Ophthalmology. 2011;in press.

34. Simonelli F, Zarrilli F, Mazzeo S, Verde V, Romano N, Savoia M, Testa F, Vitale DF, Rinaldi M, Sacchetti L. Serum oxidative and antioxidant parameters in a group of Italian patients with age-related maculopathy. Clin Chim Acta. 2002;320:111-5.

35. Blumenkranz MS, Russell SR, Robey MG, Kott-Blumenkranz R, Penneys N. Risk factors in age-related maculopathy complicated by choroidal neovascularization. Ophthalmology. 1986;93:552-8.

36. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA. 1994;272:1413-20.

37. EDCCS (Eye Disease Case Control Study). Antioxidant status and neovascular age-related macular degeneration. Eye Disease Case-Control Study Group. Arch Ophthalmol. 1993;111:104-9.

38. SanGiovanni JP, Chew EY, Clemons TE, Ferris FL, 3rd, Gensler G, Lindblad AS, Milton RC, Seddon JM, Sperduto RD. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. Arch Ophthalmol. 2007 Sep;125:1225-32.

39. Chiu CJ, Milton RC, Klein R, Gensler G, Taylor A. Dietary compound score and risk of age-related macular degeneration in the age-related eye disease study. Ophthalmology. 2009 May;116:939-46.

40. Goldberg J, Flowerdew G, Smith E, Brody JA, Tso MOM. Factors associated with age-related macular degeneration. Am JEpidemiol. 1988;128:700-10.

41. Delcourt C, Cristol JP, Tessier F, Leger CL, Descomps B, Papoz L. Age-related macular degeneration and antioxidant status in the POLA study. POLA Study Group. Pathologies Oculaires Liees a l'Age. Arch Ophthalmol. 1999;117:1384-90.

42. Fletcher AE, Bentham GC, Agnew M, Young IS, Augood C, Chakravarthy U, de Jong PT, Rahu M, Seland J, et al. Sunlight exposure, antioxidants, and age-related macular degeneration. Arch Ophthalmol. 2008 Oct;126:1396-403.

43. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. Ophthalmology. 2008 Feb;115:334-41.

44. Christen WG. Antioxidant vitamins and age-related eye disease. Proc Assoc Am Physicians. 1999;111:16-21.

45. Mares-Perlman J, Klein R, Klein BEK, Greger JL, Brady WE, Palta M, Ritter LL. Association of zinc and antioxidant nutrients with age-related maculopathy. Arch Ophthalmol. 1996;114:991-7.

46. Klein BE, Knudtson MD, Lee KE, Reinke JO, Danforth LG, Wealti AM, Moore E, Klein R. Supplements and age-related eye conditions the beaver dam eye study. Ophthalmology. 2008 Jul;115:1203-8.

47. Flood V, Smith W, Wang JJ, Manzi F, Webb K, Mitchell P. Dietary antioxidant intake and incidence of early age-related maculopathy: the Blue Mountains Eye Study. Ophthalmology. 2002;109:2272-8.

48. Cho E, Seddon JM, Rosner B, Willett WC, Hankinson SE. Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. Arch Ophthalmol. 2004;122:883-92.

49. van Leeuwen R, Boekhoorn S, Vingerling JR, Witteman JC, Klaver CC, Hofman A, de Jong PT. Dietary intake of antioxidants and risk of age-related macular degeneration. Jama. 2005 Dec 28;294:3101-7.

50. West S, Vitale S, Hallfrisch J, Munoz B, Muller D, Bressler S, Bressler NM. Are antioxidants or supplements protective for age-related macular degeneration? Arch Ophthalmol. 1994;112:222-7.

51. Belda JI, Roma J, Vilela C, Puertas FJ, Diaz-Llopis M, Bosch-Morell F, Romero FJ. Serum vitamin E levels negatively correlate with severity of age-related macular degeneration. Mechanisms of Ageing & Development. 1999;107:159-64.

52. Ishihara N, Yuzawa M, Tamakoshi A. Antioxidants and angiogenetic factor associated with age-related macular degeneration (exudative type). Nippon Ganka Gakkai Zasshi. 1997;101:248-51.

53. Michikawa T, Ishida S, Nishiwaki Y, Kikuchi Y, Tsuboi T, Hosoda K, Ishigami A, Iwasawa S, Nakano M, Takebayashi T. Serum antioxidants and age-related macular degeneration among older Japanese. Asia Pac J Clin Nutr. 2009;18:1-7.

54. Morris MS, Jacques PF, Chylack LT, Hankinson SE, Willett WC, Hubbard LD, Taylor A. Intake of zinc and antioxidant micronutrients and early age-related maculopathy lesions. Ophthalmic Epidemiol. 2007 Sep-Oct;14:288-98.

55. Mares-Perlman JA, Brady WE, Klein R, Klein BE, Bowen P, Stacewicz-Sapuntzakis M, Palta M. Serum antioxidants and age-related macular degeneration in a population-based case-control study. Arch Ophthalmol. 1995;113:1518-23.

56. Sanders TAB, Haines AP, Wormald R, Wright LA, Obeid O. Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. Am J Clin Nutr. 1993;57:428-33.

57. Smith W, Mitchell P, Rochester C. Serum beta carotene, alpha tocopherol, and age-related maculopathy: the Blue Mountains Eye Study. Am J Ophthalmol. 1997;124:838-40.

58. Cardinault N, Abalain JH, Sairafi B, Coudray C, Grolier P, Rambeau M, Carre JL, Mazur A, Rock E. Lycopene but not lutein nor zeaxanthin decreases in serum and lipoproteins in age-related macular degeneration patients. Clin Chim Acta. 2005;357:34-42.

59. Teikari JM, Laatikainen L, Virtamo J, Haukka J, Rautalahti M, Liesto K, Albanes D, Taylor P, Heinonen OP. Six-year supplementation with alpha-tocopherol and beta-carotene and age-related maculopathy. Acta Ophthalmol Scand. 1998;76:224-9.

60. Taylor HR, Tikellis G, Robman LD, McCarty CA, McNeil JJ. Vitamin E supplementation and macular degeneration: randomised controlled trial. Bmj. 2002 Jul 6;325:11.

61. The Complications of Age-Related Macular Degeneration Prevention Trial (CAPT): rationale, design and methodology. Clin Trials. 2004 Feb;1:91-107.

62. Hopley C, Salkeld G, Wang JJ, Mitchell P. Cost utility of screening and treatment for early age related macular degeneration with zinc and antioxidants. Br J Ophthalmol. 2004 Apr;88:450-4.

63. Chiu CJ, Hubbard LD, Armstrong J, Rogers G, Jacques PF, Chylack LT, Jr., Hankinson SE, Willett WC, Taylor A. Dietary glycemic index and carbohydrate in relation to early age-related macular degeneration. Am J Clin Nutr. 2006 Apr;83:880-6.

64. Chiu CJ, Milton RC, Gensler G, Taylor A. Association between dietary glycemic index and age-related macular degeneration in nondiabetic participants in the Age-Related Eye Disease Study. Am J Clin Nutr. 2007 Jul;86:180-8.

65. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981 Mar;34:362-6.

66. Chiarelli F, de Martino M, Mezzetti A, Catino M, Morgese G, Cuccurullo F, Verrotti A. Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. J Pediatr. 1999 Apr;134:486-91.

67. Kostolanska J, Jakus V, Barak L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2009 May;22:433-42.

68. Stitt AW, Li YM, Gardiner TA, Bucala R, Archer DB, Vlassara H. Advanced glycation end products (AGEs) co-localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. Am J Pathol. 1997 Feb;150:523-31.

69. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersole de Strihou C, Monnier VM, Witztum JL, Kurokawa K. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. J Clin Invest. 1997 Dec 15;100:2995-3004.

70. Dei R, Takeda A, Niwa H, Li M, Nakagomi Y, Watanabe M, Inagaki T, Washimi Y, Yasuda Y, et al. Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease. Acta Neuropathol. 2002 Aug;104:113-22.

71. Jono T, Kimura T, Takamatsu J, Nagai R, Miyazaki K, Yuzuriha T, Kitamura T, Horiuchi S. Accumulation of imidazolone, pentosidine and N(epsilon)-(carboxymethyl)lysine in hippocampal CA4 pyramidal neurons of aged human brain. Pathol Int. 2002 Sep;52:563-71.

72. Bar KJ, Franke S, Wenda B, Muller S, Kientsch-Engel R, Stein G, Sauer H. Pentosidine and N(epsilon)-(carboxymethyl)-lysine in Alzheimer's disease and vascular dementia. Neurobiol Aging. 2003 Mar-Apr;24:333-8.

73. Girones X, Guimera A, Cruz-Sanchez CZ, Ortega A, Sasaki N, Makita Z, Lafuente JV, Kalaria R, Cruz-Sanchez FF. N epsilon-carboxymethyllysine in brain aging, diabetes mellitus, and Alzheimer's disease. Free Radic Biol Med. 2004 May 15;36:1241-7.

74. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, et al. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem. 1995 Nov;65:2146-56.

75. Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF, Kowall N. Oxidative damage in Alzheimer's. Nature. 1996 Jul 11;382:120-1.

76. Munch G, Thome J, Foley P, Schinzel R, Riederer P. Advanced glycation endproducts in ageing and Alzheimer's disease. Brain Res Brain Res Rev. 1997 Feb;23:134-43.

77. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology. 2005 Jul;15:16R-28R.

78. Makita Z, Yanagisawa K, Kuwajima S, Bucala R, Vlassara H, Koike T. The role of advanced glycosylation end-products in the pathogenesis of atherosclerosis. Nephrol Dial Transplant. 1996;11 Suppl 5:31-3.

79. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovasc Res. 1998 Mar;37:586-600.

80. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001 Feb;44:129-46.

81. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. J Clin Invest. 1997 Feb 1;99:457-68.

82. Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, Koike T, Wakayama I, Yanagihara R, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol. 1998 Oct;153:1149-55.

83. Brownlee M. Glycation products and the pathogenesis of diabetic complications. Diabetes Care. 1992 Dec;15:1835-43.

84. Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S, Van Ypersele de Strihou C, Kurokawa K. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. J Am Soc Nephrol. 1998 Sep;9:1681-8.

85. Kilhovd BK, Giardino I, Torjesen PA, Birkeland KI, Berg TJ, Thornalley PJ, Brownlee M, Hanssen KF. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. Metabolism. 2003 Feb;52:163-7.

86. Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Hanssen KF, Laakso M. Increased serum levels of methylglyoxal-derived hydroimidazolone-AGE are associated with increased cardiovascular disease mortality in nondiabetic women. Atherosclerosis. 2009 Aug;205:590-4.

87. Fosmark DS, Berg JP, Jensen AB, Sandvik L, Agardh E, Agardh CD, Hanssen KF. Increased retinopathy occurrence in type 1 diabetes patients with increased serum levels of the advanced glycation endproduct hydroimidazolone. Acta Ophthalmol. 2009 Aug;87:498-500.

88. Han Y, Randell E, Vasdev S, Gill V, Curran M, Newhook LA, Grant M, Hagerty D, Schneider C. Plasma advanced glycation endproduct, methylglyoxal-derived hydroimidazolone is elevated in young, complication-free patients with Type 1 diabetes. Clin Biochem. 2009 May;42:562-9.

89. Semba RD, Fink JC, Sun K, Windham BG, Ferrucci L. Serum carboxymethyl-lysine, a dominant advanced glycation end product, is associated with chronic kidney disease: the Baltimore longitudinal study of aging. J Ren Nutr. 2010 Mar;20:74-81.

90. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. J Am Geriatr Soc. 2009 Oct;57:1874-80.

91. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, et al. Drusen proteome analysis: an approach to the etiology

of age-related macular degeneration. Proc Natl Acad Sci U S A. 2002 Nov 12;99:14682-7.

92. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem. 1998;67:425-79.

93. Luque FA, Jaffe SL. The molecular and cellular pathogenesis of dementia of the Alzheimer's type an overview. Int Rev Neurobiol. 2009;84:151-65.

94. Bisaglia M, Tessari I, Mammi S, Bubacco L. Interaction Between alpha-Synuclein and Metal Ions, Still Looking for a Role in the Pathogenesis of Parkinson's Disease. Neuromolecular Med. 2009 Aug 8.

Chapter 2. Review of the Literature

2.1 Pathogenesis of Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is a chronic disease of the retina that can ultimately result in loss of central vision (1). The retina lines the posterior section of the eye and contains a variety of cell types arranged in layers, (photoreceptors, amacrine cells, bipolar cells, horizontal cells, and ganglion cells) which convert incoming light into electrical signals that are sent along the optic nerve to the brain for visual perception (**Figures 1, 2**) (2). This conversion process, phototransduction, occurs predominantly in the photoreceptors, of which there are two types, rods and cones. Rods are distributed throughout the entire retina, and largely responsible for distinguishing dark from light. Cones are concentrated in the central, foveal region of the retina, and responsible for color vision and visual acuity. The foveal and peri-foveal regions comprise the macula. In addition to maintaining visual acuity, this region is also responsible for phototransduction of light entering from the central field of view. AMD pathology, which can result in a loss of visual acuity and central vision, is observed in the macula (1, 2).

Posterior to the photoreceptors lie the retinal pigment epithelial (RPE) cells. RPE cells facilitate transport of nutrients from the choroidal blood supply, which is posterior to the RPE, to photoreceptor outer segments (OS) (**Figure 2**) (3). This is crucial since photoreceptors do not have their own blood supply. In order to enter the RPE from the choroid, nutrients must cross Bruch's membrane, a pentalaminar structure composed of several layers of elastic and collagen (4,

5). The RPE cells also maintain retinal health by phagocytosing and digesting 10% of photoreceptor outer segments that are shed each night (2). The continual renewal of photoreceptors helps maintain retinal function in the presence of very high levels of stress in the form of light and oxygen (6). Damage to the RPE plays an important role in the development and progression of AMD (7). Disruption of RPE basal infoldings (which may aid in RPE nutrient absorption) and thickening of Bruch's membrane are thought to impair the flux of molecules into and out of the retina (8). Without proper nutrition, phagocytosis of outer segments may become impaired, resulting in an accumulation of lipofuscin in the RPE.

Lipofuscin is a lipid-based autofluorescent pigment that is one of the by-products of incomplete photoreceptor degradation (9). In addition to serving as an indicator of RPE dysfunction, this pigment also acts as a photosensitizer that produces reactive oxygen species. Thus, accumulation of lipofuscin can be both a result and a cause of RPE cellular stress (10-12).

The combination of inadequate nutrition with the inability to properly degrade and dispose of cellular debris may also contribute to the formation of deposits in the RPE-Bruch's membrane region. Basal laminar deposits are those which accumulate in the RPE cell, between the RPE basement membrane and the RPE plasma membrane. Basal linear deposits accumulate between the RPE basal lamina and the inner collagenous layer of Bruch's membrane (13). As the health of the RPE deteriorates, basal laminar and linear deposits accumulate,

and are thought to precede the formation of drusen, the clinical indicators for AMD diagnosis (14-17).

Drusen are extracellular deposits composed of lipids, proteins, and inflammatory mediators that increase in size and number as the health of the macula worsens (18). Clinicians diagnose patients with early AMD based upon the size, number and appearance (hard or soft) of drusen (14). Early AMD is indicated by small (<63 μm) and/or a few medium-sized (<125 μm) drusen. The accumulation of drusen and increased severity of pigment abnormalities increase risk for progression to the later stages of AMD (19, 20). Late AMD can manifest in two forms, Geographic Atrophy (dry AMD) and Neovascular (wet) AMD. Geographic Atrophy is characterized by atrophy of RPE cells and degeneration of the choriocapillaris (the inner vascular layers of the choroid), which leads to death of photoreceptors (**Figure 3A**). Neovascular AMD is a condition in which aberrant blood vessels develop from the choroid and penetrate into the sub-retinal space, disrupting the structural and functional integrity of the RPE and the photoreceptors it supports (**Figure 3B**). These vessels have a high risk of rupturing, which would result in bleeding in the macula. Neovascular AMD is the most visually debilitating form of the disease, and arresting this vessel growth is the target of currently available drug therapies (21). It is unclear how the extent of atrophy relates to risk for neovascularization (22).

2.2 Risk Factors for AMD

There are a variety of genetic and environmental factors that increase risk for AMD. Genome-wide analysis studies have identified many inflammatory, extracellular matrix, and mitochondrial genes that may modulate AMD risk (23). For many of these genes, the relationship between a particular gene and risk for AMD can vary between studies depending upon the population studied, the individual single nucleotide polymorphism (SNP) under analysis, and the type of AMD for which risk is being assessed (24, 25). However, variants of several genes have consistently shown to increase AMD risk.

The first genetic variant to be identified as a risk factor for AMD was the Y402H variant of the complement factor H (CFH) gene. Mutations of this gene have been associated with increased risk for neovascular AMD in several different cohorts (26-30). The CFH gene is located on the 1q31-32 chromosome, and its protein negatively regulates the alternative pathway of complement activation. Inflammation is thought to play an important role in AMD pathology, and dysfunction of this protein may lead to an uncontrollable inflammatory response that contributes to retinal damage (31). Other SNPs on the CFH gene such as rs12144939 and rs403846 have also been associated with small increases in risk for neovascular AMD (27).

In addition to CFH, two genes located on a different chromosome, 10q26, are also strongly associated with AMD risk. High-temperature requirement factor A1 (HTRA1) is a gene that encodes for a serine protease that is hypothesized to be involved in maintenance of Bruch’s membrane (32, 33). Another gene located on the 10q26 chromosome, Pleckstrin Homology Domain-containing Protein

Family A member 1/age-related maculopathy, LOC387715/ARMS2, encodes for a mitochondrial outer membrane protein that is expressed in the retina. Because mitochondria are intimately involved in energy metabolism and regulation of oxidative stress, proper mitochondrial function is required for cellular homeostasis (34). Dysfunction of the mitochondria has been demonstrated in several neurodegenerative diseases, and mitochondrial damage has been observed in AMD patients as well (35, 36). Meta-analysis of populations from North America, Europe and Asia of the rs11200638 SNP on HTRA1, and the rs10409024 SNP on LOC387715/ARMS2 revealed that each of these gene variants is associated with nearly a 3-fold increase in risk for AMD (25, 33, 37-40).

The chromosomes on which the CFH, HTRA1, and LOC387715/ARMS2 genes are located are thought to confer the most risk for AMD, but other genes involved in the complement pathway have also shown to modulate AMD risk, albeit to a smaller extent. A SNP located on the CFH receptor 5 gene, rs10922153, and rs641153 on the complement factor B gene have been shown to increase risk for neovascular AMD (27). Conversely, several genetic variants involved in complement activation appear to be protective against AMD. Rs2274700 on CFH, rs1409153 on CFH receptor 4, rs1750311 on CFH receptor 5, rs2230199 on complement component 3, rs9332739 and rs547154 on complement component 2 and rs4151667 and rs641153 on complement factor B are associated with protective effects with regard to neovascular AMD risk in a

meta-analysis of 4 cohorts, confirming the importance of the complement pathway in AMD pathology (27, 41).

Additional studies have linked variants in genes for apolipoprotein E, adenosine-triphosphate-binding transporter 4, toll-like receptor 4, and vascular endothelial growth factor to AMD risk, but the associations between these gene variants and AMD risk were not as strong as that of gene variants on chromosomes 1q31-32 and 10q26 (42-45). Collectively, these genetic studies provide insight to help explain the early observation of increased risk for AMD among primary relatives of AMD patients. Further research is needed to understand how individual SNPs affect one another and how these genetic variants are affected by other risk factors for AMD (23, 25).

Environmental factors also significantly influence risk for AMD. The strongest non-genetic risk factor for AMD is smoking, which has been shown to increase risk up to 7-fold (46). Age is also a significant risk factor (47, 48). Compared to those under the age of 40, it is estimated that in people aged 66-74 there are 10% more cases of advanced AMD, and among people aged 70-74, there are 60% more cases of advanced AMD (47). Women also have a higher risk for developing AMD (OR = 1.3), as do Caucasians compared to non-Hispanic Blacks and Hispanics (27, 49-52). However, there is a higher rate of AMD among Asians than Caucasians (52, 53). Obesity is a significant risk factor for both forms of AMD and it has been shown that a 3% reduction in waist-hip ratio decreases risk for AMD by 29% (54-56). Hypertension is thought to increase the risk for wet AMD due to increased strain on the vascular system, but

consistent epidemiological data supporting this theory is lacking (51, 57-60). Other potential risk factors such as iris color, height and hyperopia have not been consistently associated with AMD risk across different longitudinal studies (57, 61, 62).

Dietary analyses have identified additional modifiable risk factors for AMD. At first, most of these investigations focused on micronutrients, especially those that are thought to function as antioxidants (63). Vitamins C and E were two of the first nutrients studied for the potential protection they may confer on eyes, but collectively, the observational data does not indicate that individually they play a strong role in decreasing risk for AMD (63-69). A randomized, double-blinded intervention, the Age-Related Eye Disease Study (AREDS), indicated that a combination of vitamins and carotenoids, vitamins C, E, beta-carotene, zinc and copper, could slow progression of AMD from the intermediate to the later stages of disease (70). Observational data also suggest that lutein and zeaxanthin may reduce risk for neovascular AMD, but intervention trials are in progress to verify these data (71-74).

As compared with micronutrient intake, observational data supporting relationships between intake of certain macronutrients and risk for AMD are stronger. Consumption of high levels omega-3 fatty acids, especially eicosapentaenoic and docosahexanoic acids, is consistently associated with reduced risk for early as well as late AMD (75-77). Data regarding the potential mechanisms by which these long-chain fatty acids exert biological benefit suggest that omega-3 fatty acids have an anti-inflammatory effect that can

ameliorate conditions such as cardiovascular disease and AMD that are characterized by inflammation (78-81).

2.3 Dietary Glycemic Index Modulates AMD Risk: Epidemiologic Findings

Observational epidemiologic studies indicate that intake of high glycemic index (GI) carbohydrates is associated with an increased risk for onset and progression of AMD. The GI is a characteristic of a food that reflects the rise in blood glucose induced by consumption of 50 grams of carbohydrate of that particular food. Specifically, the GI of a food is a relative measure, compared to the rise in blood glucose levels induced by consumption of 50 grams of carbohydrate from a standard food such as pure glucose or white bread (82). A cross-sectional analysis of AREDS indicated that compared to people consuming diets with a GI in the first quintile (low GI), those with a dietary GI in the fourth (OR = 1.31; 95% CI: 1.02, 1.66) or fifth quintile (OR = 1.42; 95% CI: 1.09, 1.84) (high GI) were at an increased risk for the appearance of large drusen, an indicator that precedes the development of advanced AMD (83). Trend analysis showed that for each endpoint, higher risk for that particular endpoint was positively related to GI. Thus, for prevalence of large drusen $p = 0.001$, neovascular AMD $p = 0.005$, and risk for progression through the stages of AMD $p < 0.001$(83).

Prospective analysis of this same cohort confirmed the relationship between a high GI diet and increased risk for AMD. Over an 8-year period, risk for AMD progression was higher in those with a higher GI diet (RR = 1.10; 95%

CI: 1.00, 1.20; p = 0.047) (84). This association was strongest at the later stages of AMD (p < 0.001) (84). Compared to those consuming diets in the lowest quintile of GI, those consuming diets in the highest quintile of GI had 39% higher risk of progressing to advanced AMD (RR= 1.39 95% CI: 1.08, 1.79) (84). Overall, the authors predicted that reduction of dietary GI in those subjects whose GI was above the population median would decrease new cases of advanced AMD by 7.8% in 5 years (84). Furthermore, it was calculated that the AMD risk reduction associated with consumption of a low GI diet would augment the reduction in risk associated with consumption of high amounts of docosahexaenoic acid (85). This emphasizes the benefits of a healthy diet, rather than just a single nutrient.

Prospective analysis of a subset of the Nurses' Health Study confirmed the associations found in AREDS. After multivariate adjustment, women who consumed diets with a GI in the highest tertile, compared to the lowest tertile, had an increased risk for AMD (OR = 2.71; 95% CI: 1.24, 5.93; p = 0.01 for trend) (86).

The Blue Mountains Eye Study also showed that after adjusting for known biological and dietary confounders, compared to those people consuming diets in the lowest quartile of GI, those with a dietary GI in the highest quartile were at an increased risk for early AMD (RR = 1.67; 95% CI: 1.06, 2.64; p = 0.04 for trend) 10 years after baseline analyses (87). The authors went on to examine the effects of cereal fiber and low GI breads and cereals (whole-meal, mixed-grain bread, oatmeal) on risk for early AMD. It was found that there was a significant

trend of decreasing risk for early AMD with increased consumption of cereal fiber ($p = 0.05$) and low GI breads and grains ($p = 0.03$) (87).

Indicators of intermediate AMD, such as indistinct soft drusen and pigment abnormalities, were also considered endpoints in the Blue Mountains Eye Study. Compared to those who consumed diets with the lowest GI, those consuming diets in the highest quartile of GI showed an increased risk for the appearance of soft drusen over 10 years (RR = 1.68; 95% CI: 1.03, 2.74; $p = 0.04$ for trend) (87). As might be anticipated, those consuming the highest amounts of cereal fiber (compared to the lowest) also had a reduced risk of soft drusen appearance (RR = 0.61; 95% CI: 0.39, 0.96; $p = 0.01$ for trend) and pigment abnormalities (RR = 0.61; 95% CI: 0.43, 0.85; $p = 0.04$ for trend) (87). Higher consumption of low GI breads and cereals also reduced risk for soft drusen appearance (RR = 0.53; 95% CI: 0.33, 0.83; $p = 0.04$ for trend) and pigment abnormalities (RR = 0.69; 95% CI: 0.49, 0.97; $p = 0.04$ for trend). No associations were observed with early or late AMD and carbohydrate amount or intake of dietary fiber (87).

The metabolic stresses that follow consumption of high GI diets are similar (although less severe) to those observed in diabetics (88, 89). Therefore, additional support for the increased risk for AMD upon consumption of high GI diets is found in observations of higher risk for early (OR = 1.87; 95% CI: 1.07, 3.28) and neovascular AMD (OR = 1.81; 95% CI: 1.10, 2.98) among diabetics relative to non-diabetics (90, 91).

Despite the robust epidemiologic record that relates intake of lower GI diets with prolonged retinal health, there is no mechanistic information supporting

this association. Analysis of the GI-AMD relationship in a biological system is needed to confirm these epidemiological findings as well as explore the mechanism by which consumption of low GI diets may reduce AMD risk. Such studies are also relevant to understanding relationships between consuming lower GI diets and other major age-related diseases that respond to lowering GI such as diabetes, cardiovascular disease, and kidney disease (92-101). These mechanistic data will provide rationale for intervention trials which may support public health policy advocating consumption of lower GI diets for health preservation.

2.4 Animal Models of AMD

Evaluation of the role of low GI diets in reducing disease risk requires a suitable disease model. A considerable challenge in developing mouse models of AMD is that mice do not have a macula. Nevertheless, many features of human AMD can be modeled in mice. Genetic modifications are often used to elicit the pathology observed in human patients with late AMD (102). For example, knockout of the abcr, Rdh8, and Rdh12 genes results in retinal degeneration and photoreceptor atrophy (103, 104). Knocking out the Ccl2 and Ccr2 genes, which play important roles in macrophage recruitment, have been shown to induce choroidal neovascularization in some mice (105-107). Similarly, a double knockout of Ccl2 and Cx3Cr1 leads to the development of a variety of retinal lesions including photoreceptor degeneration and choroidal neovascularization in 15% of mice (108). Genetic modification of the

ceruloplasmin and hepheastin genes also results in photoreceptor atrophy and choroidal neovascularization (109). Mice that express mutant forms of ELOVL4 (Elongation of very long chain fatty acids-4) or a mutated form of the human cathepsin D gene show photoreceptor and RPE degeneration (110-112). Knockout of the very-low-density lipoprotein receptor (VLDLR) in mice also results in retinal neovascularization (113). In mice fed a high cholesterol diet, those expressing human ApoE4 develop sub-retinal deposits, pigment abnormalities, RPE atrophy, and a few mice experience choroidal neovascularization (114). These models are potential platforms for the development of late AMD treatments since they induce severe stresses in young mice within the first year. However, most of these mutations were not detected naturally and were created in the laboratory for the purpose of modeling disease. Also, the rapid, severe development of the disease phenotype in these animals leaves little time for an environmental intervention such as lowering the GI of the diet.

In contrast to the many late AMD models, there are only a limited number of early AMD rodent models. Although human forms of AMD are classified based upon their drusen size, there are no established mouse models for early or late AMD that reproducibly report drusen formation in mice (115). Therefore, determination of early AMD in mice can be made by evaluating other retinal changes that accompany drusen formation in the human condition of early AMD (116, 117). One such model of early AMD expresses human apoB100 in mice and results in the formation of basal laminar deposits, loss of basal infoldings,

and loss of fenestrations in the choriocapillaris endothelium (118-120). Early AMD can also be induced without genetic modification by feeding 16-month-old mice a high fat diet (121, 122). Espinosa-Heidmann et al. showed that feeding chow diets containing 0.8% hydroquinone (HQ) to 16-month-old mice for 4.5 months results in the formation of sub-retinal deposits, membranous debris associated with those deposits, loss of basal infoldings, thickening of Bruch's membrane, and loss of fenestrations in the choriocapillaris endothelium (123).

HQ is an oxidizing agent found at low levels in foods such as coffee, red wine, and various fruits and vegetables (124). HQ is inhaled in cigarette smoke, and in the past was applied topically as a component of a variety of cosmetics (125, 126). In cell culture systems, HQ treatment can induce oxidative and inflammatory responses, damaging mitochondria as well as DNA (127-135). The majority of the toxicity studies concerning HQ focus on renal consequences since this organ has shown to be the most sensitive to its deleterious effects. Certain strains of rodents are more susceptible to HQ-induced damage, as indicated by nephrotoxicity and renal tumors in Swiss albino mice and Eker rats exposed to very high doses of HQ (136-140). These strains of mice are especially prone to developing nephropathy. Rather than inducing nephropathy, it is thought that HQ may damage the kidney by increasing the severity of nephropathy that is already present (141).

HQ does not have nephrotoxic effects in the kidneys of C57BL/6 mice, but does contribute to the appearance of early AMD lesions (123). Although cell culture studies indicate that high doses of HQ (400 μM) cause RPE cell death,

exposures of RPE of up to 100 µM HQ in the media induce the formation of membrane blebs. Such membrane blebs are thought to contribute to the formation of sub-retinal deposits (142, 143). In addition to bleb formation, HQ exposure also up-regulates expression of CD46, CD55, and CD59 complement pathway regulatory proteins, suggesting that in addition to promoting oxidation, HQ may induce early AMD pathology through inflammatory pathways (144).

After considering the assets and difficulties of the various published models of early AMD, the model which incorporates HQ into the mouse chow was chosen for a GI intervention. Compared to the profound stress of a genetic manipulation, carbohydrate-induced stress is likely to compromise retinal integrity in a milder, more chronic manner. For analysis of the role of dietary carbohydrates in the development of early AMD, it would be best to use a model that exerts enough stress to induce early signs of AMD such as basal laminar deposits and thickening of Bruch's membrane, but not be so strong that a dietary effect would be too limited to detect and measure. The HQ model uses wild-type mice, which alleviates the concern that a genetic manipulation, such as the expression of human apoB100, would diminish the visibility of a dietary effect. Importantly, incorporation of HQ into the diet (i.e. as opposed to high amounts of fat) allows for a significant proportion of carbohydrate in the diet, maximizing the chances of observing a dietary effect of GI on the appearance of AMD-like lesions.

2.5 Molecular Mechanisms of AMD

Laboratory and human studies indicate that AMD is a multifaceted disease involving oxidative stress and inflammation (26-30, 41, 145-152). In fact, oxidized proteins and inflammatory mediators have been identified in drusen, and the size, appearance, and number of drusen are associated with RPE damage and predict AMD progress (18, 153). However, a full understanding of the mechanisms that contribute to AMD is still lacking.

Oxidative insult is thought to be one of the main sources of retinal stress. Photoreceptors are saturated with polyunsaturated fatty acids that are metabolized to lipid peroxides under oxidative conditions (154-157). These lipid peroxides damage photoreceptor membranes as well as RPE membranes, which can contribute to the formation of basal laminar deposits (8, 158). Increases in lipid peroxidation in the macula are seen not only with experimental models of oxidative stress such as blue-light exposure, but also with increasing age (159). Additionally, oxidation of lipoproteins can trigger oxidative stress, inflammation, and apoptosis in RPE cells, as well as impair degradation of photoreceptor outer segments (160, 161).

The phagocytic turnover of photoreceptors by the RPE is an inherently oxidative process that results in the formation of lipofuscin, a lipid-protein aggregate (162). Lipofuscin accumulates with age as well as under conditions of oxidative stress or RPE damage (154, 163-165). Although an early event in the pathogenesis of AMD, lipofuscin accumulation and RPE damage as measured by fundus autofluorescence has been associated with decreased visual function (166). Lipofuscin enters the RPE along with A2E, a pigment formed from

phosphatidylethanolamine and all-trans retinal in photoreceptors. A2E is autofluorescent and is most known for its role as a photosensitizer, a compound that produces reactive oxygen species after reacting with oxygen (10-12, 167). Recent data also indicates that A2E prevents cholesterol from leaving the RPE cell (168). This causes cholesteryl esters to accumulate and displaces cholesterol from cell membranes. Cholesterol deposits have been found under the RPE and in drusen of patients with AMD (169-171). Thus, in addition to generating oxidative stress, lipofuscin and A2E may contribute to AMD by impairing cholesterol metabolism.

In addition to directly damaging the retina, oxidative stress also modulates expression of vascular endothelial growth factor (VEGF) and pigment epithelium derived factor (PEDF), two growth factors which regulate angiogenesis in neovascular AMD (172-174). Oxidative regulation of these growth factors is thought to be mediated by endoplasmic reticulum stress which is increased in oxidative conditions and induces expression of VEGF (175-181). The role of oxidative stress in neovascularization is also supported by results from several mouse studies (172, 182, 183). In a VEGF-induced model of retinopathy, mice that lacked superoxide dismutase-1, a key antioxidant enzyme, experienced a greater level of neovascularization than wild type mice (182). Another study showed that compared to wild type mice, mice lacking a subunit of NADPH oxidase, an enzyme that generates reaction oxygen species, had reduced neovascularization following laser exposure (183).

The oxidation-induced retinal damage observed in these mechanistic studies is consistent with results from population cohort studies, as indicated by the markedly increased risk for AMD found in smokers (184). Further evidence of the role of oxidative stress in disease pathogenesis is found in the ability of the AREDS supplement, an antioxidant cocktail, to protect against progression to late-stage AMD (21). Furthermore, it has also been noted that the antioxidant activities of glutathione peroxidase and superoxide dismutase in the RPE not only decrease during aging, but are lower in AMD patients compared to healthy controls (185-189).

Oxidative insults to the retina may result in inefficient control of, or chronic activation of inflammatory responses (190-192). A variety of inflammatory mediators such as C5b-9, vitronectin and MHC class II antigens have been identified on drusen, suggesting that inflammation is involved in the pathogenesis of early AMD (193, 194). Dendritic cells have also been identified in drusen, and some studies suggest that oxidatively-stressed RPE cells secrete cytokines such as monocyte chemoattractant protein-1, and interleukin-8 to initiate an inflammatory response and recruit these dendritic cells to the RPE (153, 195-200). Further evidence of such an inflammatory response during AMD is found in the observation of macrophages in Bruch's membrane as well as activated microglia in the retinas of AMD patients (201, 202).

In the late stages of AMD, neovascularization appears to be governed largely by pro-inflammatory growth factors such as VEGF (203-205). The role of VEGF is profound. Treatments that inhibit the activity of VEGF slow vision loss

in neovascular AMD patients (206-208). Other inflammatory mediators such as hepatocyte growth factor, platelet-derived growth factor, fibroblast growth factor 2 and interleukin-10 have been shown to induce cellular processes involved in choroidal neovascularization such as endothelial cell proliferation and migration and macrophage polarization (209-215).

Collectively, these data indicate that oxidative stress and inflammation may be involved in both early and late AMD. Different types of oxidative stress (reactive oxygen species, lipid peroxides, oxidized lipoproteins) and several different inflammatory pathways (complement, macrophage recruitment, dendritic recruitment) appear to be involved in retinal aging and pathology, but the specific sequence of events is not known.

2.6 Glycative Stress: Another Mechanism of AMD

Advanced glycation end products (AGEs) are formed when proteins react with glucose, glucose metabolites such as methylglyoxal, or other glycating agents such as reactive dicarbonyls in a Maillard reaction (**Figure 4**) (216). These non-enzymatic reactions can occur in the presence or absence of oxidative stress, and the exact conditions under which the protein and glycating agent interact determines the individual structure of the AGE (217). Serum levels of AGEs have been associated with increased age, as well as diabetes, neurodegenerative diseases, kidney disease and cardiovascular disease (218-226).

Analogous to the identification of oxidized lipoproteins and pro-inflammatory mediators in drusen, the presence of AGEs in drusen, basal laminar deposits, basal linear deposits, Bruch's membrane and in RPE cells suggests that these modified proteins may also be involved in the retinal damage that accompanies AMD (8, 18, 227). Importantly, retinal deposition of AGEs is greater in AMD patients compared to age-matched controls, suggesting that AGEs are not simply an indicator of aging, but of disease (18, 228).

The health of the RPE depends upon unimpeded flow of nutrients into the RPE from the choroid, across Bruch's membrane. The ability of AGEs to trap macromolecules, combined with their deposition in drusen, basal laminar or linear deposits, and Bruch's membrane, suggests that they may impair trafficking of materials into and out of the RPE (229, 230). This could create a stressful environment in the RPE, similar to that which precedes AMD (231). In corroboration of this concept, when RPE cells are grown in the presence of AGEs, there is an increase in Bruch's membrane thickness, suggesting that nutrient traffic may be disrupted (232). The presence of AGEs in RPE cells also increases expression of cytokines associated with choroidal neovascularization, indicating that AGEs may influence inflammatory stress pathways involved in AMD pathogenesis (227).

Mechanistic studies in other tissues such as the kidney, vascular tissue and brain have begun to shed light on the association between AGEs and pathology in these organs. The kidney is one of the organs responsible for clearance of AGEs, and AGEs are most commonly found in the kidney when

kidney function is compromised such as in diabetes or in chronic kidney disease. Since the kidney is at the end of the AGE metabolic pathway, the levels of AGEs found in the kidney reflect not only renal stress, but whole-body AGE stress (233, 234).

Accumulation of AGEs in the heart has been associated with decreased heart function and increased risk for cardiovascular disease (235). An etiological role for AGEs is indicated in that AGEs decrease levels of nitric oxide and promote vascular stiffness, a component of cardiovascular disease (236-240). Accumulation of AGEs is also related to neurodegenerative disease. In neuroblastoma cells, introduction of AGEs causes an increase in aggregation of amyloid beta and impairs nerve regeneration, which could explain the elevated levels of AGEs and subsequent nerve damage in Alzheimer's disease patients (241, 242).

In addition to these tissue-specific effects of AGEs in disease, the inflammatory processes that accompany chronic disease may also facilitate AGE accumulation. For example, it has been observed that diabetics and patients with metabolic syndrome have lower levels of endogenously secreted receptor for advanced glycation end products (esRAGE), than healthy controls (243-245). Therefore, these patients cannot clear AGEs as effectively, and AGEs may accumulate in the blood. Once AGEs do interact with their receptor at the plasma membrane, a series of oxidative and inflammatory pathways are activated within the cell (246-254). In addition to the RAGE pathway, several studies also indicate that AGE-induced endoplasmic reticulum stress may

potentiate oxidative stress through activation of the unfolded protein response (177, 178, 246, 253-261). Taken together, these studies indicate that AGEs have the potential to create a stressful cellular environment and increase susceptibility to disease. However, it is not clear if they are causally associated with AMD and other chronic diseases. For AMD, if AGEs are causative factors in disease progression, those factors which are responsible for their accumulation in the retina need to be elucidated.

2.7 Glycemic Index as a Modulator of AGEs

The formation of AGEs requires glucose, or a glucose-derivative. It is therefore not surprising that diabetes, a condition of poor glycemic control and increased blood glucose levels, was one of the first diseases associated with AGE accumulation (218, 220). It has been shown that improvement of glycemic control in diabetics is associated with a decrease in serum AGEs, suggesting that the duration and amount of sugar present in the blood directly affects the accumulation of AGEs (262, 263).

Despite the association between consumption of high GI diets and increased risk for diseases such as cardiovascular disease, renal disease and AMD (that are also associated with AGE accumulation) there is very little data concerning the effect GI modulation on accumulation of intracellular AGEs within tissues (83, 84, 86, 92-101). The only mechanistic study available indicates that feeding diabetic rats a low GI food reduces the accumulation of AGEs in the kidney (264). More documentation and understanding of the effects of high and

low GI diets on AGE accumulation in other tissues such as the eye, heart and brain will indicate if the effects of dietary GI modulation are systemic. These data will begin to elucidate whether the intracellular stresses evoked by high or low GI diets are related to disease risk. Importantly, an understanding of the effect of dietary GI on AGE accumulation in the retina may help to explain the epidemiological data linking high GI diets with increased risk for AMD (84, 86).

2.8 Formation vs. Degradation of AGEs

AGEs may enter the cell through receptors such as RAGE, or may be formed intracellularly following facilitated diffusion of glucose into the cell via glucose transporters, such as GLUTs. There are several different isoforms of GLUTs, some of which are only expressed in certain tissues. GLUTs 1 and 3 are expressed in all cell types (including retina, lens, brain, vascular endothelium), except the liver, intestine, and pancreatic beta cells (265-267). GLUT 2 is mainly expressed in the liver, kidney, intestine, and in beta cells of the pancreas. GLUT 4 is found in the heart, adipose, and skeletal muscle (268-272). In addition to these well-characterized Class I GLUTs, these tissues also express a variety of Class II and III GLUTs (272). Binding affinity for glucose is similar across the different isoforms, except for GLUT 2 which has a lower affinity for glucose. This explains why glucose uptake in the liver may be slower than in other tissues, but continues after the other isoforms have been saturated (273). Although there is not very much published information regarding the effect of AGEs on GLUT

expression, it has been shown in bovine retinal endothelial cells that AGE exposure does not alter GLUT 1 expression (274).

Following GLUT-mediated transport of glucose, AGEs can be formed from reactive dicarbonyls such as glyoxal, 3-deoxyglucosone and methylglyoxal. Glyoxal is produced from the auto-oxidation of glucose, 3-deoxyglucosone is produced following decomposition of Amadori products, and methylglyoxal can be formed from glyceraldehydes-3-phosphate and dihydroxyacetone phosphate (275-277). These carbonyls then react with proteins to form AGEs, such as when methylglyoxal reacts with arginine residues of proteins to form 5-hydro-5-methylimidazolone (MG-H1)-modified proteins (**Figure 5**). MG-H1-modified protein is one of the most biologically abundant AGEs (278, 279). Once AGEs are formed in the cell they can exert damage by altering the function of the proteins that they have modified, as well as disrupting interactions of extracellular matrix proteins (280).

AGEs are also substrates of intracellular protein degradation pathways (Uchiki et al, 2011 *in press*). One of the major pathways responsible for intracellular protein quality control is the ubiquitin proteasome system (UPS). Ubiquitin is a 76 amino acid protein that when bound to a protein substrate can facilitate degradation of that substrate by the proteasome. Ubiquitin is first activated by a ubiquitin activating enzyme (E1) which binds to ubiquitin via a covalent thiol ester linkage. Ubiquitin is then transferred to a ubiquitin conjugating enzyme (E2) to form another thiol ester bond. The E2 may transfer ubiquitin to a ubiquitin ligase (E3) which then transfers ubiquitin to the substrate.

Alternatively, the E3 may serve as a scaffold between the E2 and the substrate, allowing the direct transfer of ubiquitin from the E2 to the substrate. This cascade is repeated until the substrate has at least 4 ubiquitin moieties attached, at which point it can be recognized by the proteasome for degradation. After the substrate is degraded, the ubiquitin moieties are removed from substrate and recycled for further use (**Figure 6**) (281).

There are no publications that indicate how consuming diets of different GI modulates the UPS. However, glycative stress has been modeled *in vitro*. Thus, glycative stress inhibited ubiquitination of substrates, impaired activity of ubiquitin conjugating enzymes, but surprisingly did not affect activity of the proteasome (Uchiki et al, 2011, *in press*). AGE-modified proteins also appear to be less susceptible to UPS-mediated degradation in cell-free and cell culture systems than un-modified proteins (Uchiki et al, 2011 *in press*). It remains to be determined how glycative stress induced by dietary GI affects the UPS in animal models, and if this can explain intracellular AGE accumulation.

2.9 Summary of the Literature

AMD impairs the vision and quality of life of millions of elderly Americans. Despite recent advances in the development of drugs to slow vision loss, the majority of those diagnosed do not have an effective pharmacologic treatment option. Epidemiological data suggests that specific dietary regimens, such as the consumption of a low GI diet and adequate intake of omega-3 fatty acids and antioxidants can significantly decrease the risk of AMD onset and progression.

My work focuses on confirming the salutary effects of consuming lower GI diets in an intervention study and beginning to clarify the mechanism by which they diminish risk for AMD.

Investigation of the effects of GI on AMD requires an animal model of early AMD that is sensitive to a subtle, chronic stress and in which the effects of carbohydrate modification can be measured. Of the current known AMD models, the dietary HQ model provides the best context for analysis of GI on AMD. Similar to other AMD models, this model is thought to induce signs of early AMD through both oxidative and inflammatory processes. Using a GI intervention in this model, we will be able to explore the role of dietary GI as well as AGEs in the pathogenesis of AMD.

Though AGE accumulation has been associated with a variety of chronic diseases, the reason for this accumulation is not clear. Therefore, an investigation of the effects of consuming high and low GI diets on early AMD, AGE accumulation in tissues, and alteration of the UPS will provide insight into the biological mechanism underlying the association between GI and AMD. These data would also help characterize the potential roles of dietary GI and AGEs in pathology of other chronic diseases.

2.10 References

1. National Eye Institute NIoH. Facts About Age-Related Macular Degeneration. 2010.

2. Sung CH, Chuang JZ. The cell biology of vision. J Cell Biol. 2010 Sep 20;190:953-63.

3. Zarbin MA. Age-related macular degeneration: review of pathogenesis. Eur J Ophthalmol. 1998 Oct-Dec;8:199-206.

4. Sivaprasad S, Bailey TA, Chong VN. Bruch's membrane and the vascular intima: is there a common basis for age-related changes and disease? Clin Experiment Ophthalmol. 2005 Oct;33:518-23.

5. Shakib M, Zinn KM. Fine structure and function of ocular tissues. The choroid, Bruch's membrane, and the retinal pigment epithelium. Int Ophthalmol Clin. 1973 Fall;13:189-204.

6. Young RW. The renewal of photoreceptor cell outer segments. J Cell Biol. 1967 Apr;33:61-72.

7. Cai J, Nelson KC, Wu M, Sternberg PJ, Jones DP. Oxidative damage and protection of the RPE. Prog Retin Eye Res. 2000;19:205-21.

8. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol. 2004;122:598-614.

9. Fujihara M, Nagai N, Sussan TE, Biswal S, Handa JT. Chronic cigarette smoke causes oxidative damage and apoptosis to retinal pigmented epithelial cells in mice. PLoS ONE. 2008;3:e3119.

10. Delmelle M. Retinal sensitized photodynamic damage to liposomes. Photochem Photobiol. 1978 Sep;28:357-60.

11. Gaillard ER, Atherton SJ, Eldred G, Dillon J. Photophysical studies on human retinal lipofuscin. Photochem Photobiol. 1995 May;61:448-53.

12. Rozanowska M, Jarvis-Evans J, Korytowski W, Boulton ME, Burke JM, Sarna T. Blue light-induced reactivity of retinal age pigment. In vitro generation of oxygen-reactive species. J Biol Chem. 1995 Aug 11;270:18825-30.

13. Curcio CA, Millican CL. Basal linear deposit and large drusen are specific for early age-related maculopathy. Arch Ophthalmol. 1999 Mar;117:329-39.

14. Ding X, Patel M, Chan CC. Molecular pathology of age-related macular degeneration. Prog Retin Eye Res. 2009 Jan;28:1-18.

15. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. N Engl J Med. 2008 Jun 12;358:2606-17.

16. Al-Hussaini H, Schneiders M, Lundh P, Jeffery G. Drusen are associated with local and distant disruptions to human retinal pigment epithelium cells. Exp Eye Res. 2009 Mar;88:610-2.

17. Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, Neufeld AH. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. PLoS ONE. 2009;4:e4160.

18. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci U S A. 2002 Nov 12;99:14682-7.

19. Smiddy WE, Fine SL. Prognosis of patients with bilateral macular drusen. Ophthalmology. 1984 Mar;91:271-7.

20. Davis MD, Gangnon RE, Lee LY, Hubbard LD, Klein BE, Klein R, Ferris FL, Bressler SB, Milton RC. The Age-Related Eye Disease Study severity scale

for age-related macular degeneration: AREDS Report No. 17. Arch Ophthalmol. 2005 Nov;123:1484-98.

21. Group A-REDSR. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001 Oct;119:1417-36.

22. Sunness JS, Gonzalez-Baron J, Bressler NM, Hawkins B, Applegate CA. The development of choroidal neovascularization in eyes with the geographic atrophy form of age-related macular degeneration. Ophthalmology. 1999 May;106:910-9.

23. Katta S, Kaur I, Chakrabarti S. The molecular genetic basis of age-related macular degeneration: an overview. J Genet. 2009 Dec;88:425-49.

24. Charbel Issa P, Chong NV, Scholl HP. The significance of the complement system for the pathogenesis of age-related macular degeneration - current evidence and translation into clinical application. Graefes Arch Clin Exp Ophthalmol. 2011 Feb;249:163-74.

25. Tong Y, Liao J, Zhang Y, Zhou J, Zhang H, Mao M. LOC387715/HTRA1 gene polymorphisms and susceptibility to age-related macular degeneration: A HuGE review and meta-analysis. Mol Vis. 2010;16:1958-81.

26. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005;308:419-21.

27. Hageman GS, Gehrs K, Lejnine S, Bansal AT, Deangelis MM, Guymer RH, Baird PN, Allikmets R, Deciu C, et al. Clinical validation of a genetic model to estimate the risk of developing choroidal neovascular age-related macular degeneration. Hum Genomics. 2011 Jul 1;5:420-40.

28. Wegscheider BJ, Weger M, Renner W, Steinbrugger I, Marz W, Mossbock G, Temmel W, El-Shabrawi Y, Schmut O, et al. Association of complement factor H Y402H gene polymorphism with different subtypes of exudative age-related macular degeneration. Ophthalmology. 2007 Apr;114:738-42.

29. Souied EH, Leveziel N, Richard F, Dragon-Durey MA, Coscas G, Soubrane G, Benlian P, Fremeaux-Bacchi V. Y402H complement factor H polymorphism associated with exudative age-related macular degeneration in the French population. Mol Vis. 2005;11:1135-40.

30. Thakkinstian A, Han P, McEvoy M, Smith W, Hoh J, Magnusson K, Zhang K, Attia J. Systematic review and meta-analysis of the association between complement factor H Y402H polymorphisms and age-related macular degeneration. Hum Mol Genet. 2006 Sep 15;15:2784-90.

31. Pettigrew HD, Teuber SS, Gershwin ME. Clinical significance of complement deficiencies. Ann N Y Acad Sci. 2009 Sep;1173:108-23.

32. Oka C, Tsujimoto R, Kajikawa M, Koshiba-Takeuchi K, Ina J, Yano M, Tsuchiya A, Ueta Y, Soma A, et al. HtrA1 serine protease inhibits signaling mediated by Tgfbeta family proteins. Development. 2004 Mar;131:1041-53.

33. Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, et al. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science. 2006 Nov 10;314:992-3.

34. Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med. 2000 May;6:513-9.

35. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature. 2006 Oct 19;443:787-95.

36. Feher J, Kovacs I, Artico M, Cavallotti C, Papale A, Balacco Gabrieli C. Mitochondrial alterations of retinal pigment epithelium in age-related macular degeneration. Neurobiol Aging. 2006 Jul;27:983-93.

37. Leveziel N, Souied EH, Richard F, Barbu V, Zourdani A, Morineau G, Zerbib J, Coscas G, Soubrane G, Benlian P. PLEKHA1-LOC387715-HTRA1 polymorphisms and exudative age-related macular degeneration in the French population. Mol Vis. 2007;13:2153-9.

38. Lin JM, Wan L, Tsai YY, Lin HJ, Tsai Y, Lee CC, Tsai CH, Tsai FJ, Tseng SH. HTRA1 polymorphism in dry and wet age-related macular degeneration. Retina. 2008 Feb;28:309-13.

39. Kondo N, Honda S, Ishibashi K, Tsukahara Y, Negi A. LOC387715/HTRA1 variants in polypoidal choroidal vasculopathy and age-related macular degeneration in a Japanese population. Am J Ophthalmol. 2007 Oct;144:608-12.

40. Kanda A, Chen W, Othman M, Branham KE, Brooks M, Khanna R, He S, Lyons R, Abecasis GR, Swaroop A. A variant of mitochondrial protein

LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci U S A. 2007 Oct 9;104:16227-32.

41. Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat Genet. 2006 Apr;38:458-62.

42. Baird PN, Richardson AJ, Robman LD, Dimitrov PN, Tikellis G, McCarty CA, Guymer RH. Apolipoprotein (APOE) gene is associated with progression of age-related macular degeneration (AMD). Hum Mutat. 2006 Apr;27:337-42.

43. Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science. 1997 Sep 19;277:1805-7.

44. Zareparsi S, Buraczynska M, Branham KE, Shah S, Eng D, Li M, Pawar H, Yashar BM, Moroi SE, et al. Toll-like receptor 4 variant D299G is associated with susceptibility to age-related macular degeneration. Hum Mol Genet. 2005 Jun 1;14:1449-55.

45. Churchill AJ, Carter JG, Lovell HC, Ramsden C, Turner SJ, Yeung A, Escardo J, Atan D. VEGF polymorphisms are associated with neovascular age-related macular degeneration. Hum Mol Genet. 2006 Oct 1;15:2955-61.

46. Klein R, Cruickshanks KJ, Nash SD, Krantz EM, Javier Nieto F, Huang GH, Pankow JS, Klein BE. The prevalence of age-related macular degeneration and associated risk factors. Arch Ophthalmol. Jun;128:750-8.

47. Friedman DS, O'Colmain BJ, Munoz B, Tomany SC, McCarty C, de Jong PT, Nemesure B, Mitchell P, Kempen J, Group. EDPR. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol. 2004;122:564-72.

48. Klein R, Cruickshanks KJ, Nash SD, Krantz EM, Javier Nieto F, Huang GH, Pankow JS, Klein BE. The prevalence of age-related macular degeneration and associated risk factors. Arch Ophthalmol. 2010 Jun;128:750-8.

49. Frank RN, Puklin JE, Stock C, Canter LA. Race, iris color, and age-related macular degeneration. Trans Am Ophthalmol Soc. 2000;98:109-15; discussion 15-7.

50. Klein R, Klein BE, Marino EK, Kuller LH, Furberg C, Burke GL, Hubbard LD. Early age-related maculopathy in the cardiovascular health study. Ophthalmology. 2003;110:25-33.

51. Cruickshanks KJ, Hamman RF, Klein R, Nondahl DM, Shetterly SM. The prevalence of age-related maculopathy by geographic region and ethnicity. The Colorado-Wisconsin Study of Age-Related Maculopathy. Arch Ophthalmol. 1997 Feb;115:242-50.

52. Klein R. Race/Ethnicity and age-related macular degeneration. Am J Ophthalmol. 2011 Aug;152:153-4.

53. Klein R, Klein BE, Knudtson MD, Wong TY, Cotch MF, Liu K, Burke G, Saad MF, Jacobs DRJ. Prevalence of age-related macular degeneration in 4 racial/ethnic groups in the multi-ethnic study of atherosclerosis. Ophthalmology. 2006;113:373-80.

54. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. Am J Ophthalmol. 2004;137:486-95.

55. Peeters A, Magliano DJ, Stevens J, Duncan BB, Klein R, Wong TY. Changes in abdominal obesity and age-related macular degeneration: the Atherosclerosis Risk in Communities Study. Arch Ophthalmol. 2008 Nov;126:1554-60.

56. Klein BE, Klein R, Lee KE, Jensen SC. Measures of obesity and age-related eye diseases. Ophthalmic Epidemiol. 2001 Sep;8:251-62.

57. Klein R. Overview of progress in the epidemiology of age-related macular degeneration. Ophthalmic Epidemiol. 2007 Jul-Aug;14:184-7.

58. Risk factors for choroidal neovascularization in the second eye of patients with juxtafoveal or subfoveal choroidal neovascularization secondary to age-related macular degeneration. Macular Photocoagulation Study Group. Arch Ophthalmol. 1997 Jun;115:741-7.

59. Klein R, Klein BEK, Linton KLP. Prevalence of age-related maculopathy. Opthalmology. 1992;99:933-43.

60. Hawkins BS, Bird A, Klein R, West SK. Epidemiology of age-related macular degeneration. Mol Vis. 1999;5:26.

61. Mitchell P, Smith W, Wang JJ. Iris color, skin sun sensitivity, and age-related maculopathy. The Blue Mountains Eye Study. Ophthalmology. 1998 Aug;105:1359-63.

62. Fraser-Bell S, Choudhury F, Klein R, Azen S, Varma R. Ocular risk factors for age-related macular degeneration: the Los Angeles Latino Eye Study. Am J Ophthalmol. May;149:735-40.

63. Weikel K, Taylor, A. Nutritional Modulation of Cataract and Age-Related Macular Degeneration. Duanes Ophthalmology. 2011;in press.

64. Goldberg J, Flowerdew G, Smith E, Brody JA, Tso MOM. Factors associated with age-related macular degeneration. Am JEpidemiol. 1988;128:700-10.

65. Cho E, Seddon JM, Rosner B, Willett WC, Hankinson SE. Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. Arch Ophthalmol. 2004;122:883-92.

66. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein BE, Brady WE, Palta M. Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. Am J Epidemiol. 1998;148:204-14.

67. Sanders TAB, Haines AP, Wormald R, Wright LA, Obeid O. Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. Am J Clin Nutr. 1993;57:428-33.

68. Delcourt C, Cristol JP, Tessier F, Leger CL, Descomps B, Papoz L. Age-related macular degeneration and antioxidant status in the POLA study. POLA Study Group. Pathologies Oculaires Liees a l'Age. Arch Ophthalmol. 1999;117:1384-90.

69. Tan AG, Mitchell P, Flood VM, Burlutsky G, Rochtchina E, Cumming RG, Wang JJ. Antioxidant nutrient intake and the long-term incidence of age-related cataract: the Blue Mountains Eye Study. Am J Clin Nutr. 2008 Jun;87:1899-905.

70. Chew EY, Lindblad AS, Clemons T. Summary results and recommendations from the age-related eye disease study. Arch Ophthalmol. 2009 Dec;127:1678-9.

71. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA. 1994;272:1413-20.

72. SanGiovanni JP, Chew EY, Clemons TE, Ferris FL, 3rd, Gensler G, Lindblad AS, Milton RC, Seddon JM, Sperduto RD. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. Arch Ophthalmol. 2007 Sep;125:1225-32.

73. Cho E, Hankinson SE, Rosner B, Willett WC, Colditz GA. Prospective study of lutein/zeaxanthin intake and risk of age-related macular degeneration. Am J Clin Nutr. 2008 Jun;87:1837-43.

74. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. Ophthalmology. 2008 Feb;115:334-41.

75. Chua B, Flood V, Rochtchina E, Wang JJ, Smith W, Mitchell P. Dietary fatty acids and the 5-year incidence of age-related maculopathy. Arch Ophthalmol. 2006 Jul;124:981-6.

76. Tan JS, Wang JJ, Flood V, Mitchell P. Dietary fatty acids and the 10-year incidence of age-related macular degeneration: the Blue Mountains Eye Study. Arch Ophthalmol. 2009 May;127:656-65.

77. Augood C, Chakravarthy U, Young I, Vioque J, de Jong PT, Bentham G, Rahu M, Seland J, Soubrane G, et al. Oily fish consumption, dietary docosahexaenoic acid and eicosapentaenoic acid intakes, and associations with neovascular age-related macular degeneration. Am J Clin Nutr. 2008 Aug;88:398-406.

78. Schweigert FJ, Reimann J. Micronutrients and their Relevance for the Eye - Function of Lutein, Zeaxanthin and Omega-3 Fatty Acids. Klin Monbl Augenheilkd. 2011;228:537-43.

79. Pilkington SM, Watson RE, Nicolaou A, Rhodes LE. Omega-3 polyunsaturated fatty acids: photoprotective macronutrients. Exp Dermatol. 2011;20:537-43.

80. Sapieha P, Stahl A, Chen J, Seaward MR, Willett KL, Krah NM, Dennison RJ, Connor KM, Aderman CM, et al. 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids. Sci Transl Med. Feb 9;3:69ra12.

81. Bacova B, Radosinska J, Knezl V, Kolenova L, Weismann P, Navarova J, Barancik M, Mitasikova M, Tribulova N. Omega-3 fatty acids and atorvastatin

suppress ventricular fibrillation inducibility in hypertriglyceridemic rat hearts: implication of intracellular coupling protein, connexin-43. J Physiol Pharmacol. Dec;61:717-23.

82. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981 Mar;34:362-6.

83. Chiu CJ, Milton RC, Gensler G, Taylor A. Association between dietary glycemic index and age-related macular degeneration in nondiabetic participants in the Age-Related Eye Disease Study. Am J Clin Nutr. 2007 Jul;86:180-8.

84. Chiu CJ, Milton RC, Klein R, Gensler G, Taylor A. Dietary carbohydrate and the progression of age-related macular degeneration: a prospective study from the Age-Related Eye Disease Study. Am J Clin Nutr. 2007 Oct;86:1210-8.

85. Chiu CJ, Klein R, Milton RC, Gensler G, Taylor A. Does eating particular diets alter risk of age-related macular degeneration in users of the age-related eye disease study supplements? Br J Ophthalmol. 2009 Jun 9:1241-6.

86. Chiu CJ, Hubbard LD, Armstrong J, Rogers G, Jacques PF, Chylack J, L. T., Hankinson SE, Willett WC, Taylor A. Dietary glycemic index and carbohydrate in relation to early age-related macular degeneration. Am J Clin Nutr. 2006;83:880-6.

87. Kaushik S, Wang JJ, Flood V, Tan JS, Barclay AW, Wong TY, Brand-Miller J, Mitchell P. Dietary glycemic index and the risk of age-related macular degeneration. Am J Clin Nutr. 2008 Oct;88:1104-10.

88. Benhalima K, Standl E, Mathieu C. The importance of glycemic control: how low should we go with HbA1c? Start early, go safe, go low. J Diabetes Complications. 2011 May-Jun;25:202-7.

89. Marsh K, Barclay A, Colagiuri S, Brand-Miller J. Glycemic index and glycemic load of carbohydrates in the diabetes diet. Curr Diab Rep. 2011 Apr;11:120-7.

90. Choi JK, Lym YL, Moon JW, Shin HJ, Cho B. Diabetes mellitus and early age-related macular degeneration. Arch Ophthalmol. 2011 Feb;129:196-9.

91. Topouzis F, Anastasopoulos E, Augood C, Bentham GC, Chakravarthy U, de Jong PT, Rahu M, Seland J, Soubrane G, et al. Association of diabetes with age-related macular degeneration in the EUREYE study. Br J Ophthalmol. 2009 Aug;93:1037-41.

92. Ferland A, Brassard P, Lemieux S, Bergeron J, Bogaty P, Bertrand F, Simard S, Poirier P. Impact of high-fat /low-carbohydrate, high-, low-glycaemic index or low-caloric meals on glucose regulation during aerobic exercise in Type 2 diabetes. Diabet Med. 2009 Jun;26:589-95.

93. Jenkins DJ, Kendall CW, McKeown-Eyssen G, Josse RG, Silverberg J, Booth GL, Vidgen E, Josse AR, Nguyen TH, et al. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. Jama. 2008 Dec 17;300:2742-53.

94. McKeown NM, Meigs JB, Liu S, Rogers G, Yoshida M, Saltzman E, Jacques PF. Dietary carbohydrates and cardiovascular disease risk factors in the Framingham offspring cohort. J Am Coll Nutr. 2009 Apr;28:150-8.

95. Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH, Willett WC. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr. 1999;70:412-9.

96. Riegersperger M, Sunder-Plassmann G. How to prevent progression to end stage renal disease. J Ren Care. 2007 Jul-Sep;33:105-7.

97. Gopinath B, Harris DC, Flood VM, Burlutsky G, Brand-Miller J, Mitchell P. Carbohydrate nutrition is associated with the 5-year incidence of chronic kidney disease. J Nutr. 2011 Mar;141:433-9.

98. Semba RD, Fink JC, Sun K, Bandinelli S, Guralnik JM, Ferrucci L. Carboxymethyl-lysine, an advanced glycation end product, and decline of renal function in older community-dwelling adults. Eur J Nutr. 2009 Feb;48:38-44.

99. Jenkins DJ, Srichaikul K, Kendall CW, Sievenpiper JL, Abdulnour S, Mirrahimi A, Meneses C, Nishi S, He X, et al. The relation of low glycaemic index fruit consumption to glycaemic control and risk factors for coronary heart disease in type 2 diabetes. Diabetologia. 2011 Feb;54:271-9.

100. Chiu C-J, Liu, S., Willett, W. C., Wolever, T.M.S., Brand-Miller, J. C. Barclay, A. C., A. Taylor. Informing food choices and health outcomes by use of the dietary glycemic index Nutrition Reviews. 2011 April;69:231-42.

101. Chiu CJ, Taylor A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. Prog Retin Eye Res. 2011 Jan;30:18-53.

102. Rakoczy EP, Yu MJ, Nusinowitz S, Chang B, Heckenlively JR. Mouse models of age-related macular degeneration. Exp Eye Res. 2006 May;82:741-52.

103. Maeda A, Golczak M, Maeda T, Palczewski K. Limited roles of Rdh8, Rdh12, and Abca4 in all-trans-retinal clearance in mouse retina. Invest Ophthalmol Vis Sci. 2009 Nov;50:5435-43.

104. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell. 1999 Jul 9;98:13-23.

105. Radu RA, Mata NL, Bagla A, Travis GH. Light exposure stimulates formation of A2E oxiranes in a mouse model of Stargardt's macular degeneration. Proc Natl Acad Sci U S A. 2004 Apr 20;101:5928-33.

106. Mata NL, Weng J, Travis GH. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. Proc Natl Acad Sci U S A. 2000 Jun 20;97:7154-9.

107. Ambati J, Anand A, Fernandez S, Sakurai E, Lynn BC, Kuziel WA, Rollins BJ, Ambati BK. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat Med. 2003 Nov;9:1390-7.

108. Tuo J, Bojanowski CM, Zhou M, Shen D, Ross RJ, Rosenberg KI, Cameron DJ, Yin C, Kowalak JA, et al. Murine ccl2/cx3cr1 deficiency results in retinal lesions mimicking human age-related macular degeneration. Invest Ophthalmol Vis Sci. 2007 Aug;48:3827-36.

109. Hahn P, Qian Y, Dentchev T, Chen L, Beard J, Harris ZL, Dunaief JL. Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. Proc Natl Acad Sci U S A. 2004 Sep 21;101:13850-5.

110. Karan G, Lillo C, Yang Z, Cameron DJ, Locke KG, Zhao Y, Thirumalaichary S, Li C, Birch DG, et al. Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant ELOVL4 transgenic mice: a model for macular degeneration. Proc Natl Acad Sci U S A. 2005 Mar 15;102:4164-9.

111. Rakoczy PE, Zhang D, Robertson T, Barnett NL, Papadimitriou J, Constable IJ, Lai CM. Progressive age-related changes similar to age-related macular degeneration in a transgenic mouse model. Am J Pathol. 2002 Oct;161:1515-24.

112. Zhang D, Lai MC, Constable IJ, Rakoczy PE. A model for a blinding eye disease of the aged. Biogerontology. 2002;3:61-6.

113. Heckenlively JR, Hawes NL, Friedlander M, Nusinowitz S, Hurd R, Davisson M, Chang B. Mouse model of subretinal neovascularization with choroidal anastomosis. Retina. 2003 Aug;23:518-22.

114. Malek G, Johnson LV, Mace BE, Saloupis P, Schmechel DE, Rickman DW, Toth CA, Sullivan PM, Bowes Rickman C. Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration. Proc Natl Acad Sci U S A. 2005 Aug 16;102:11900-5.

115. Imamura Y, Noda S, Hashizume K, Shinoda K, Yamaguchi M, Uchiyama S, Shimizu T, Mizushima Y, Shirasawa T, Tsubota K. Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. Proc Natl Acad Sci U S A. 2006 Jul 25;103:11282-7.

116. Sarks S, Cherepanoff S, Killingsworth M, Sarks J. Relationship of Basal laminar deposit and membranous debris to the clinical presentation of early age-related macular degeneration. Invest Ophthalmol Vis Sci. 2007 Mar;48:968-77.

117. Sarks SH. Ageing and degeneration in the macular region: a clinicopathologic study. Br J Ophthalmol. 1976;60:324-41.

118. Espinosa-Heidmann DG, Sall J, Hernandez EP, Cousins SW. Basal laminar deposit formation in APO B100 transgenic mice: complex interactions between dietary fat, blue light, and vitamin E. Invest Ophthalmol Vis Sci. 2004 Jan;45:260-6.

119. Callow MJ, Stoltzfus LJ, Lawn RM, Rubin EM. Expression of human apolipoprotein B and assembly of lipoprotein(a) in transgenic mice. Proc Natl Acad Sci U S A. 1994 Mar 15;91:2130-4.

120. Fujihara M, Bartels E, Nielsen LB, Handa JT. A human apoB100 transgenic mouse expresses human apoB100 in the RPE and develops features of early AMD. Exp Eye Res. 2009 Jun;88:1115-23.

121. Provost AC, Vede L, Bigot K, Keller N, Tailleux A, Jais JP, Savoldelli M, Ameqrane I, Lacassagne E, et al. Morphologic and electroretinographic phenotype of SR-BI knockout mice after a long-term atherogenic diet. Invest Ophthalmol Vis Sci. 2009 Aug;50:3931-42.

122. Cousins SW, Espinosa-Heidmann DG, Alexandridou A, Sall J, Dubovy S, Csaky K. The role of aging, high fat diet and blue light exposure in an experimental mouse model for basal laminar deposit formation. Exp Eye Res. 2002 Nov;75:543-53.

123. Espinosa-Heidmann DG, Suner IJ, Catanuto P, Hernandez EP, Marin-Castano ME, Cousins SW. Cigarette Smoke-Related Oxidants and the Development of Sub-RPE Deposits in an Experimental Animal Model of Dry AMD. Invest Ophthalmol Vis Sci. 2006 Feb;47:729-37.

124. Deisinger PJ, Hill TS, English JC. Human exposure to naturally occurring hydroquinone. J Toxicol Environ Health. 1996 Jan;47:31-46.

125. Hoffmann D, Wynder EL. Chemical constituents and bioactivity of tobacco smoke. IARC Sci Publ. 1986:145-65.

126. Andersen FA, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Jr., Shank RC, Slaga TJ, Snyder PW. Final amended safety assessment of hydroquinone as used in cosmetics. Int J Toxicol. Nov-Dec;29:274S-87.

127. Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ Health Perspect. 1997 Jun;105 Suppl 4:875-82.

128. Winston GW, Church DF, Cueto R, Pryor WA. Oxygen consumption and oxyradical production from microsomal reduction of aqueous extracts of cigarette tar. Arch Biochem Biophys. 1993 Aug 1;304:371-8.

129. Lenaz G. Quinone specificity of complex I. Biochim Biophys Acta. 1998 May 6;1364:207-21.

130. Niki E, Minamisawa S, Oikawa M, Komuro E. Membrane damage from lipid oxidation induced by free radicals and cigarette smoke. Ann N Y Acad Sci. 1993 May 28;686:29-37; discussion -8.

131. Bertram KM, Baglole CJ, Phipps RP, Libby RT. Molecular regulation of cigarette smoke induced-oxidative stress in human retinal pigment epithelial cells: implications for age-related macular degeneration. Am J Physiol Cell Physiol. 2009 Nov;297:C1200-10.

132. Andreoli C, Rossi S, Leopardi P, Crebelli R. DNA damage by hydroquinone in human white blood cells: analysis by alkaline single-cell gel electrophoresis. Mutat Res. 1999 Jan 2;438:37-45.

133. Luo L, Jiang L, Geng C, Cao J, Zhong L. Hydroquinone-induced genotoxicity and oxidative DNA damage in HepG2 cells. Chem Biol Interact. 2008 May 9;173:1-8.

134. Li Q, Geiselhart L, Mittler JN, Mudzinski SP, Lawrence DA, Freed BM. Inhibition of human T lymphoblast proliferation by hydroquinone. Toxicol Appl Pharmacol. 1996 Aug;139:317-23.

135. Lee JY, Kim JY, Lee YG, Shin WC, Chun T, Rhee MH, Cho JY. Hydroquinone, a reactive metabolite of benzene, reduces macrophage-mediated immune responses. Mol Cells. 2007 Apr 30;23:198-206.

136. Topping DC, Bernard LG, O'Donoghue JL, English JC. Hydroquinone: acute and subchronic toxicity studies with emphasis on neurobehavioral and nephrotoxic effects. Food Chem Toxicol. 2007 Jan;45:70-8.

137. Boatman RJ, English JC, Perry LG, Bialecki VE. Differences in the nephrotoxicity of hydroquinone among Fischer 344 and Sprague-Dawley rats and B6C3F1 mice. J Toxicol Environ Health. 1996 Feb 9;47:159-72.

138. Jagetia GC, Aruna R. Hydroquinone increases the frequency of micronuclei in a dose-dependent manner in mouse bone marrow. Toxicol Lett. 1997 Dec;93:205-13.

139. Lau SS, Monks TJ, Everitt JI, Kleymenova E, Walker CL. Carcinogenicity of a nephrotoxic metabolite of the "nongenotoxic" carcinogen hydroquinone. Chem Res Toxicol. 2001 Jan;14:25-33.

140. English JC, Hill T, O'Donoghue JL, Reddy MV. Measurement of nuclear DNA modification by 32P-postlabeling in the kidneys of male and female Fischer 344 rats after multiple gavage doses of hydroquinone. Fundam Appl Toxicol. 1994 Oct;23:391-6.

141. Andersen FA, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Jr., Shank RC, Slaga TJ, Snyder PW. Final amended safety assessment of hydroquinone as used in cosmetics. Int J Toxicol. 2010 Nov-Dec;29:274S-87.

142. Marin-Castano ME, Striker GE, Alcazar O, Catanuto P, Espinosa-Heidmann DG, Cousins SW. Repetitive Nonlethal Oxidant Injury to Retinal Pigment Epithelium Decreased Extracellular Matrix Turnover In Vitro and Induced Sub-RPE Deposits In Vivo. Invest Ophthalmol Vis Sci. 2006 Sep;47:4098-112.

143. Pons M, Cousins SW, Csaky KG, Striker G, Marin-Castano ME. Cigarette smoke-related hydroquinone induces filamentous actin reorganization and heat shock protein 27 phosphorylation through p38 and extracellular signal-regulated

kinase 1/2 in retinal pigment epithelium: implications for age-related macular degeneration. Am J Pathol. 2010 Sep;177:1198-213.

144. Yang P, Tyrrell J, Han I, Jaffe GJ. Expression and modulation of RPE cell membrane complement regulatory proteins. Invest Ophthalmol Vis Sci. 2009 Jul;50:3473-81.

145. Edwards AO, Ritter R, 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005 Apr 15;308:421-4.

146. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci U S A. 2005 May 17;102:7227-32.

147. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005 Apr 15;308:419-21.

148. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005 Apr 15;308:385-9.

149. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am J Hum Genet. 2005 Sep;77:389-407.

150. Spencer KL, Hauser MA, Olson LM, Schmidt S, Scott WK, Gallins P, Agarwal A, Postel EA, Pericak-Vance MA, Haines JL. Protective effect of complement factor B and complement component 2 variants in age-related macular degeneration. Hum Mol Genet. 2007 Aug 15;16:1986-92.

151. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat Genet. 2007 Oct;39:1200-1.

152. Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, Clayton DG, Hayward C, Morgan J, et al. Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med. 2007 Aug 9;357:553-61.

153. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res. 2001;20:705-32.

154. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol. 2000 Sep-Oct;45:115-34.

155. Bazan NG. The metabolism of omega-3 polyunsaturated fatty acids in the eye: the possible role of docosahexaenoic acid and docosanoids in retinal physiology and ocular pathology. Prog Clin Biol Res. 1989;312:95-112.

156. Ham WT, Jr., Ruffolo JJ, Jr., Mueller HA, Clarke AM, Moon ME. Histologic analysis of photochemical lesions produced in rhesus retina by short-wave-length light. Invest Ophthalmol Vis Sci. 1978 Oct;17:1029-35.

157. Wiegand RD, Giusto NM, Rapp LM, Anderson RE. Evidence for rod outer segment lipid peroxidation following constant illumination of the rat retina. Invest Ophthalmol Vis Sci. 1983 Oct;24:1433-5.

158. Anderson SM, Krinsky NI. Protective action of carotenoid pigments against photodynamic damage to liposomes. Photochem Photobiol. 1973 Nov;18:403-8.

159. De La Paz M, Anderson RE. Region and age-dependent variation in susceptibility of the human retina to lipid peroxidation. Invest Ophthalmol Vis Sci. 1992 Dec;33:3497-9.

160. Yamada Y, Tian J, Yang Y, Cutler RG, Wu T, Telljohann RS, Mattson MP, Handa JT. Oxidized low density lipoproteins induce a pathologic response by retinal pigmented epithelial cells. J Neurochem. 2008 May;105:1187-97.

161. Hoppe G, Marmorstein AD, Pennock EA, Hoff HF. Oxidized low density lipoprotein-induced inhibition of processing of photoreceptor outer segments by RPE. Invest Ophthalmol Vis Sci. 2001 Oct;42:2714-20.

162. Tate DJ, Jr., Miceli MV, Newsome DA. Phagocytosis and H2O2 induce catalase and metallothionein gene expression in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci. 1995 Jun;36:1271-9.

163. Feeney-Burns L, Hilderbrand ES, Eldridge S. Aging human RPE: morphometric analysis of macular, equatorial, and peripheral cells. Invest Ophthalmol Vis Sci. 1984 Feb;25:195-200.

164. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. Eye (Lond). 1988;2 (Pt 5):552-77.

165. Dorey CK, Wu G, Ebenstein D, Garsd A, Weiter JJ. Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. Invest Ophthalmol Vis Sci. 1989 Aug;30:1691-9.

166. Schmitz-Valckenberg S, Fleckenstein M, Scholl HP, Holz FG. Fundus autofluorescence and progression of age-related macular degeneration. Surv Ophthalmol. 2009 Jan-Feb;54:96-117.

167. Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. Exp Eye Res. 2005 May;80:595-606.

168. Lakkaraju A, Finnemann SC, Rodriguez-Boulan E. The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proc Natl Acad Sci U S A. 2007 Jun 26;104:11026-31.

169. Curcio CA, Presley JB, Millican CL, Medeiros NE. Basal deposits and drusen in eyes with age-related maculopathy: evidence for solid lipid particles. Exp Eye Res. 2005 Jun;80:761-75.

170. Li CM, Chung BH, Presley JB, Malek G, Zhang X, Dashti N, Li L, Chen J, Bradley K, et al. Lipoprotein-like particles and cholesteryl esters in human Bruch's membrane: initial characterization. Invest Ophthalmol Vis Sci. 2005 Jul;46:2576-86.

171. Malek G, Li CM, Guidry C, Medeiros NE, Curcio CA. Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. Am J Pathol. 2003 Feb;162:413-25.

172. Shen JK, Dong A, Hackett SF, Bell WR, Green WR, Campochiaro PA. Oxidative damage in age-related macular degeneration. Histol Histopathol. 2007 Dec;22:1301-8.

173. Ohno-Matsui K, Morita I, Tombran-Tink J, Mrazek D, Onodera M, Uetama T, Hayano M, Murota SI, Mochizuki M. Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. J Cell Physiol. 2001 Dec;189:323-33.

174. Spaide RF, Ho-Spaide WC, Browne RW, Armstrong D. Characterization of peroxidized lipids in Bruch's membrane. Retina. 1999;19:141-7.

175. He S, Yaung J, Kim YH, Barron E, Ryan SJ, Hinton DR. Endoplasmic reticulum stress induced by oxidative stress in retinal pigment epithelial cells. Graefes Arch Clin Exp Ophthalmol. 2008 May;246:677-83.

176. Santos CX, Tanaka LY, Wosniak J, Laurindo FR. Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. Antioxid Redox Signal. 2009 Oct;11:2409-27.

177. Gargalovic PS, Gharavi NM, Clark MJ, Pagnon J, Yang WP, He A, Truong A, Baruch-Oren T, Berliner JA, et al. The unfolded protein response is an important regulator of inflammatory genes in endothelial cells. Arterioscler Thromb Vasc Biol. 2006 Nov;26:2490-6.

178. Oskolkova OV, Afonyushkin T, Leitner A, von Schlieffen E, Gargalovic PS, Lusis AJ, Binder BR, Bochkov VN. ATF4-dependent transcription is a key

mechanism in VEGF up-regulation by oxidized phospholipids: critical role of oxidized sn-2 residues in activation of unfolded protein response. Blood. 2008 Jul 15;112:330-9.

179. Sanson M, Auge N, Vindis C, Muller C, Bando Y, Thiers JC, Marachet MA, Zarkovic K, Sawa Y, et al. Oxidized low-density lipoproteins trigger endoplasmic reticulum stress in vascular cells: prevention by oxygen-regulated protein 150 expression. Circ Res. 2009 Feb 13;104:328-36.

180. Salminen A, Kauppinen A, Suuronen T, Kaarniranta K, Ojala J. ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. J Neuroinflammation. 2009;6:41.

181. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature. 2008 Jul 24;454:455-62.

182. Dong A, Xie B, Shen J, Yoshida T, Yokoi K, Hackett SF, Campochiaro PA. Oxidative stress promotes ocular neovascularization. J Cell Physiol. 2009 Jun;219:544-52.

183. Li Q, Dinculescu A, Shan Z, Miller R, Pang J, Lewin AS, Raizada MK, Hauswirth WW. Downregulation of p22phox in retinal pigment epithelial cells inhibits choroidal neovascularization in mice. Mol Ther. 2008 Oct;16:1688-94.

184. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect. 1985 Dec;64:111-26.

185. Liles MR, Newsome DA, Oliver PD. Antioxidant enzymes in the aging human retinal pigment epithelium. Arch Ophthalmol. 1991;109:1285-8.

186. Frank RN, Amin RH, Puklin JE. Antioxidant enzymes in the macular retinal pigment epithelium of eyes with neovascular age-related macular degeneration. Am J Ophthalmol. 1999 Jun;127:694-709.

187. Tate DJ, Jr., Newsome DA, Oliver PD. Metallothionein shows an age-related decrease in human macular retinal pigment epithelium. Invest Ophthalmol Vis Sci. 1993 Jun;34:2348-51.

188. Cohen SM, Olin KL, Feuer WJ, Hjelmeland L, Keen CL, Morse LS. Low glutathione reductase and peroxidase activity in age-related macular degeneration. Br J Ophthalmol. 1994;78:791-4.

189. Delcourt C, Cristol JP, Leger CL, Descomps B, Papoz L. Associations of antioxidant enzymes with cataract and age-related macular degeneration. The POLA Study. Pathologies Oculaires Liees a l'Age. Ophthalmology. 1999 Feb;106:215-22.

190. Lau LI, Chiou SH, Liu CJ, Yen MY, Wei YH. The Effect of Photo-oxidative Stress and Inflammatory Cytokine on Complement Factor H Expression in Retinal Pigment Epithelial Cells. Invest Ophthalmol Vis Sci. 2011;52:6832-41.

191. Pons M, Marin-Castano ME. Cigarette smoke-related hydroquinone dysregulates MCP-1, VEGF and PEDF expression in retinal pigment epithelium in vitro and in vivo. PLoS One. 2011;6:e16722.

192. Libby RT, Gould DB. Endoplasmic reticulum stress as a primary pathogenic mechanism leading to age-related macular degeneration. Adv Exp Med Biol. 2011;664:403-9.

193. Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. Exp Eye Res. 2000 Apr;70:441-9.

194. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. Faseb J. 2000;14:835-46.

195. Holtkamp GM, Kijlstra A, Peek R, de Vos AF. Retinal pigment epithelium-immune system interactions: cytokine production and cytokine-induced changes. Prog Retin Eye Res. 2001 Jan;20:29-48.

196. Hageman GS, Mullins RF. Molecular composition of drusen as related to substructural phenotype. Mol Vis. 1999;5:28.

197. Mainster MA. Light and macular degeneration: a biophysical and clinical perspective. Eye (Lond). 1987;1 (Pt 2):304-10.

198. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell. 2001 Feb 23;104:503-16.

199. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999 Jan 14;340:115-26.

200. Higgins GT, Wang JH, Dockery P, Cleary PE, Redmond HP. Induction of angiogenic cytokine expression in cultured RPE by ingestion of oxidized photoreceptor outer segments. Invest Ophthalmol Vis Sci. 2003 Apr;44:1775-82.

201. Killingsworth MC, Sarks JP, Sarks SH. Macrophages related to Bruch's membrane in age-related macular degeneration. Eye (Lond). 1990;4 (Pt 4):613-21.

202. Penfold PL, Liew SC, Madigan MC, Provis JM. Modulation of major histocompatibility complex class II expression in retinas with age-related macular degeneration. Invest Ophthalmol Vis Sci. 1997 Sep;38:2125-33.

203. Lopez PF, Sippy BD, Lambert HM, Thach AB, Hinton DR. Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. Invest Ophthalmol Vis Sci. 1996 Apr;37:855-68.

204. Kvanta A, Algvere PV, Berglin L, Seregard S. Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. Invest Ophthalmol Vis Sci. 1996 Aug;37:1929-34.

205. Kliffen M, Sharma HS, Mooy CM, Kerkvliet S, de Jong PT. Increased expression of angiogenic growth factors in age-related maculopathy. Br J Ophthalmol. 1997 Feb;81:154-62.

206. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology. 2009 Jan;116:57-65 e5.

207. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006 Oct 5;355:1419-31.

208. Gragoudas ES, Adamis AP, Cunningham ET, Jr., Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med. 2004 Dec 30;351:2805-16.

209. Nakamura Y, Morishita R, Higaki J, Kida I, Aoki M, Moriguchi A, Yamada K, Hayashi S, Yo Y, et al. Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor. J Hypertens. 1996 Sep;14:1067-72.

210. Tamagnone L, Comoglio PM. Control of invasive growth by hepatocyte growth factor (HGF) and related scatter factors. Cytokine Growth Factor Rev. 1997 Jun;8:129-42.

211. Nagineni CN, Kutty V, Detrick B, Hooks JJ. Expression of PDGF and their receptors in human retinal pigment epithelial cells and fibroblasts: regulation by TGF-beta. J Cell Physiol. 2005 Apr;203:35-43.

212. Kaven C, Spraul CW, Zavazava N, Lang GK, Lang GE. Thalidomide and prednisolone inhibit growth factor-induced human retinal pigment epithelium cell proliferation in vitro. Ophthalmologica. 2001 Jul-Aug;215:284-9.

213. Rosenthal R, Malek G, Salomon N, Peill-Meininghaus M, Coeppicus L, Wohlleben H, Wimmers S, Bowes Rickman C, Strauss O. The fibroblast growth factor receptors, FGFR-1 and FGFR-2, mediate two independent signalling

pathways in human retinal pigment epithelial cells. Biochem Biophys Res Commun. 2005 Nov 11;337:241-7.

214. Hollborn M, Jahn K, Limb GA, Kohen L, Wiedemann P, Bringmann A. Characterization of the basic fibroblast growth factor-evoked proliferation of the human Muller cell line, MIO-M1. Graefes Arch Clin Exp Ophthalmol. 2004 May;242:414-22.

215. Kelly J, Ali Khan A, Yin J, Ferguson TA, Apte RS. Senescence regulates macrophage activation and angiogenic fate at sites of tissue injury in mice. J Clin Invest. 2007 Nov;117:3421-6.

216. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001 Feb;44:129-46.

217. Fu MX, Wells-Knecht KJ, Blackledge JA, Lyons TJ, Thorpe SR, Baynes JW. Glycation, glycoxidation, and cross-linking of collagen by glucose. Kinetics, mechanisms, and inhibition of late stages of the Maillard reaction. Diabetes. 1994 May;43:676-83.

218. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. J Clin Invest. 1997 Feb 1;99:457-68.

219. Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, Koike T, Wakayama I, Yanagihara R, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol. 1998 Oct;153:1149-55.

220. Brownlee M. Glycation products and the pathogenesis of diabetic complications. Diabetes Care. 1992 Dec;15:1835-43.

221. Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S, Van Ypersele de Strihou C, Kurokawa K. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. J Am Soc Nephrol. 1998 Sep;9:1681-8.

222. Kilhovd BK, Giardino I, Torjesen PA, Birkeland KI, Berg TJ, Thornalley PJ, Brownlee M, Hanssen KF. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. Metabolism. 2003 Feb;52:163-7.

223. Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Hanssen KF, Laakso M. Increased serum levels of methylglyoxal-derived hydroimidazolone-AGE are associated with increased cardiovascular disease mortality in nondiabetic women. Atherosclerosis. 2009 Aug;205:590-4.

224. Fosmark DS, Berg JP, Jensen AB, Sandvik L, Agardh E, Agardh CD, Hanssen KF. Increased retinopathy occurrence in type 1 diabetes patients with increased serum levels of the advanced glycation endproduct hydroimidazolone. Acta Ophthalmol. 2009 Aug;87:498-500.

225. Han Y, Randell E, Vasdev S, Gill V, Curran M, Newhook LA, Grant M, Hagerty D, Schneider C. Plasma advanced glycation endproduct, methylglyoxal-derived hydroimidazolone is elevated in young, complication-free patients with Type 1 diabetes. Clin Biochem. 2009 May;42:562-9.

226. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. Proc Natl Acad Sci U S A. 1994 Nov 22;91:11704-8.

227. Ishibashi T, Murata T, Hangai M, Nagai R, Horiuchi S, Lopez PF, Hinton DR, Ryan SJ. Advanced glycation end products in age-related macular degeneration. Arch Ophthalmol. 1998 Dec;116:1629-32.

228. Hammes HP, Hoerauf H, Alt A, Schleicher E, Clausen JT, Bretzel RG, Laqua H. N(epsilon)(carboxymethyl)lysin and the AGE receptor RAGE colocalize in age-related macular degeneration. Invest Ophthalmol Vis Sci. 1999;40:1855-9.

229. Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. Biochim Biophys Acta. 2000 Dec 20;1498:99-111.

230. King GL, Brownlee M. The cellular and molecular mechanisms of diabetic complications. Endocrinol Metab Clin North Am. 1996 Jun;25:255-70.

231. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol. 2004 Apr;122:598-614.

232. Honda S, Farboud B, Hjelmeland LM, Handa JT. Induction of an aging mRNA retinal pigment epithelial cell phenotype by matrix-containing advanced glycation end products in vitro. Invest Ophthalmol Vis Sci. 2001;42:2419-25.

233. Busch M, Franke S, Ruster C, Wolf G. Advanced glycation end-products and the kidney. Eur J Clin Invest. 2010 Aug;40:742-55.

234. Chougale AD, Bhat SP, Bhujbal SV, Zambare MR, Puntambekar S, Somani RS, Boppana R, Giri AP, Kulkarni MJ. Proteomic Analysis of Glycated

Proteins from Streptozotocin-Induced Diabetic Rat Kidney. Mol Biotechnol. 2011 Apr 23.

235. Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, Hanssen KF. Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. Diabetes Care. 1999 Jul;22:1186-90.

236. van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJ, Schalkwijk CG, Bronzwaer JG, et al. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. Circulation. 2008 Jan 1;117:43-51.

237. Ma H, Li SY, Xu P, Babcock SA, Dolence EK, Brownlee M, Li J, Ren J. Advanced glycation endproduct (AGE) accumulation and AGE receptor (RAGE) up-regulation contribute to the onset of diabetic cardiomyopathy. J Cell Mol Med. 2009 Aug;13:1751-64.

238. Ceriello A. Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. Diabetes Nutr Metab. 1999;12:42-6.

239. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation. 2003 Jan 7;107:139-46.

240. Lai YL, Aoyama S, Nagai R, Miyoshi N, Ohshima H. Inhibition of L-arginine metabolizing enzymes by L-arginine-derived advanced glycation end products. J Clin Biochem Nutr. 2010 Mar;46:177-85.

241. Woltjer RL, Maezawa I, Ou JJ, Montine KS, Montine TJ. Advanced glycation endproduct precursor alters intracellular amyloid-beta/A beta PP carboxy-terminal fragment aggregation and cytotoxicity. J Alzheimers Dis. 2003 Dec;5:467-76.

242. Yan SD, Chen X, Schmidt AM, Brett J, Godman G, Zou YS, Scott CW, Caputo C, Frappier T, et al. Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc Natl Acad Sci U S A. 1994 Aug 2;91:7787-91.

243. Koyama H, Shoji T, Yokoyama H, Motoyama K, Mori K, Fukumoto S, Emoto M, Shoji T, Tamei H, et al. Plasma level of endogenous secretory RAGE is associated with components of the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol. 2005 Dec;25:2587-93.

244. Katakami N, Matsuhisa M, Kaneto H, Matsuoka TA, Sakamoto K, Nakatani Y, Ohtoshi K, Hayaishi-Okano R, Kosugi K, et al. Decreased endogenous secretory advanced glycation end product receptor in type 1 diabetic patients: its possible association with diabetic vascular complications. Diabetes Care. 2005 Nov;28:2716-21.

245. Koyama H, Yamamoto H, Nishizawa Y. RAGE and soluble RAGE: potential therapeutic targets for cardiovascular diseases. Mol Med. 2007 Nov-Dec;13:625-35.

246. Makita Z, Yanagisawa K, Kuwajima S, Bucala R, Vlassara H, Koike T. The role of advanced glycosylation end-products in the pathogenesis of atherosclerosis. Nephrol Dial Transplant. 1996;11 Suppl 5:31-3.

247. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovasc Res. 1998 Mar;37:586-600.

248. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem. 1994 Apr 1;269:9889-97.

249. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM. Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. J Biol Chem. 1997 Jul 11;272:17810-4.

250. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001;108:949-55.

251. Schmidt AM, Yan SD, Brett J, Mora R, Nowygrod R, Stern D. Regulation of human mononuclear phagocyte migration by cell surface-binding proteins for advanced glycation end products. J Clin Invest. 1993 May;91:2155-68.

252. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. J Clin Invest. 1995 Sep;96:1395-403.

253. Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, Schmidt AM. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. J Clin Invest. 1996 Sep 1;98:1088-94.

254. Zong H, Ward M, Stitt AW. AGEs, RAGE, and Diabetic Retinopathy. Curr Diab Rep. 2011 May 19.

255. Che W, Asahi M, Takahashi M, Kaneto H, Okado A, Higashiyama S, Taniguchi N. Selective induction of heparin-binding epidermal growth factor-like growth factor by methylglyoxal and 3-deoxyglucosone in rat aortic smooth muscle cells. The involvement of reactive oxygen species formation and a possible implication for atherogenesis in diabetes. J Biol Chem. 1997 Jul 18;272:18453-9.

256. Yim MB, Yim HS, Lee C, Kang SO, Chock PB. Protein glycation: creation of catalytic sites for free radical generation. Ann N Y Acad Sci. 2001 Apr;928:48-53.

257. Yan H, Harding JJ. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. Biochem J. 1997 Dec 1;328 (Pt 2):599-605.

258. Obrosova IG. How does glucose generate oxidative stress in peripheral nerve? Int Rev Neurobiol. 2002;50:3-35.

259. Jiang JM, Wang Z, Li DD. Effects of AGEs on oxidation stress and antioxidation abilities in cultured astrocytes. Biomed Environ Sci. 2004 Mar;17:79-86.

260. Scivittaro V, Ganz MB, Weiss MF. AGEs induce oxidative stress and activate protein kinase C-beta(II) in neonatal mesangial cells. Am J Physiol Renal Physiol. 2000 Apr;278:F676-83.

261. Rosca MG, Mustata TG, Kinter MT, Ozdemir AM, Kern TS, Szweda LI, Brownlee M, Monnier VM, Weiss MF. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. Am J Physiol Renal Physiol. 2005 Aug;289:F420-30.

262. Chiarelli F, de Martino M, Mezzetti A, Catino M, Morgese G, Cuccurullo F, Verrotti A. Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. J Pediatr. 1999 Apr;134:486-91.

263. Kostolanska J, Jakus V, Barak L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2009 May;22:433-42.

264. Bhaskar JJ, Shobha MS, Sambaiah K, Salimath PV. Beneficial effects of banana (Musa sp. var. elakki bale) flower and pseudostem on hyperglycemia and advanced glycation end-products (AGEs) in streptozotocin-induced diabetic rats. J Physiol Biochem. 2011 Apr 8.

265. Cohen G, Riahi Y, Alpert E, Gruzman A, Sasson S. The roles of hyperglycaemia and oxidative stress in the rise and collapse of the natural protective mechanism against vascular endothelial cell dysfunction in diabetes. Arch Physiol Biochem. 2007 Oct-Dec;113:259-67.

266. Kumagai AK. Glucose transport in brain and retina: implications in the management and complications of diabetes. Diabetes Metab Res Rev. 1999 Jul-Aug;15:261-73.

267. Nakazawa Y, Oka M, Bando M, Inoue T, Takehana M. The role of ascorbic acid transporter in the lens of streptozotocin-induced diabetic rat. Biomed Pharmacother. 2010 Sep 25.

268. Takagi H, Tanihara H, Seino Y, Yoshimura N. Characterization of glucose transporter in cultured human retinal pigment epithelial cells: gene expression and effect of growth factors. Invest Ophthalmol Vis Sci. 1994 Jan;35:170-7.

269. Alexiou P, Chatzopoulou M, Pegklidou K, Demopoulos VJ. RAGE: a multi-ligand receptor unveiling novel insights in health and disease. Curr Med Chem. 2010;17:2232-52.

270. Thorens B, Mueckler M. Glucose transporters in the 21st Century. Am J Physiol Endocrinol Metab. 2010 Feb;298:E141-5.

271. Kuhla A, Hettwer C, Menger MD, Vollmar B. Oxidative stress-associated rise of hepatic protein glycation increases inflammatory liver injury in uncoupling protein-2 deficient mice. Lab Invest. 2010 Aug;90:1189-98.

272. Wood IS, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br J Nutr. 2003 Jan;89:3-9.

273. Carruthers A. Facilitated diffusion of glucose. Physiol Rev. 1990 Oct;70:1135-76.

274. Barathi S, Angayarkanni N, Sumantran VN. GLUT-1 expression in bovine retinal capillary endothelial cells and pericytes exposed to advanced glycation end products. Invest Ophthalmol Vis Sci. 2010 Dec;51:6810-4.

275. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001 Dec 13;414:813-20.

276. Wells-Knecht KJ, Zyzak DV, Litchfield JE, Thorpe SR, Baynes JW. Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose. Biochemistry. 1995 Mar 21;34:3702-9.

277. Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J. 1990 Jul 1;269:1-11.

278. Ahmed N, Argirov OK, Minhas HS, Cordeiro CA, Thornalley PJ. Assay of advanced glycation endproducts (AGEs): surveying AGEs by chromatographic assay with derivatization by 6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate and application to Nepsilon-carboxymethyl-lysine- and Nepsilon-(1-carboxyethyl)lysine-modified albumin. Biochem J. 2002 May 15;364:1-14.

279. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, Brownlee M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents

hyperglycemia-induced increases in macromolecular endocytosis. J Clin Invest. 1998 Mar 1;101:1142-7.

280. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010 Oct 29;107:1058-70.

281. Pickart CM, Eddins MJ. Ubiquitin: structures, functions, mechanisms. Biochim Biophys Acta. 2004 Nov 29;1695:55-72.

282. National Eye Institute NIoH. Eye diagram.; 2010.

283. Therapeutics O. What is Age-Related Macular Degeneration (AMD)? ; 2006.

284. Knapowski J, Wieczorowska-Tobis K, Witowski J. Pathophysiology of ageing. J Physiol Pharmacol. 2002 Jun;53:135-46.

285. Carrier L, Schlossarek S, Willis MS, Eschenhagen T. The ubiquitin-proteasome system and nonsense-mediated mRNA decay in hypertrophic cardiomyopathy. Cardiovasc Res. 2010 Jan 15;85:330-8.

2.11 Figure Legends

Figure 1. Eye diagram (282). The retina lines the posterior section of the eye. The macula is located near the center of the retina.

Figure 2. Retina cross section (2). Rods, cones, bipolar cells, horizontal cells, amacrine cells, ganglion cells and Müller cells are arranged in several layers: GCL = ganglion cell layer (ganglion cells); IPL = inner plexiform layer; INL = inner nuclear layer (Müller, amacrine, bipolar cell nuclei); IS = inner photoreceptor

segments; OPL = outer plexiform layer; ONL = outer nuclear layer (rod, cone, horizontal cell nuclei); OS = outer photoreceptor segments; RPE = retinal pigment epithelium.

Figure 3. Pathology of dry and wet AMD (283). Schematic of dry (A) and wet (B) AMD. In dry AMD, RPE atrophy leads to photoreceptor loss. In wet AMD, neovascularization disrupts RPE and increases risk for vessel leakage.

Figure 4. Formation of AGEs (284). Glucose reacts with the amine group of a protein in a Maillard reaction to ultimately form an advanced glycation end product.

Figure 5. Preparation of methylglyoxal-derived hydroimidazolones. (a) Isomer 1, MG-H1; (b) isomer 2, MG-H2; (c) isolation of isomer 3, MG-H3 (278).

Figure 6. Ubiquitin Proteasome System (UPS). The UPS is an ATP-dependent proteolytic system that requires polyubiquitination of a target protein prior to degradation by the 26S proteasome. Polyubiquitination of lysine residues involves E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin ligase) enzymes. Chains of at least 4 ubiquitin moieties can target a protein substrate for degradation by the 26S proteasome. Once the substrate is degraded, ubiquitin moieties are recycled for further use (285).

2.12 Figures

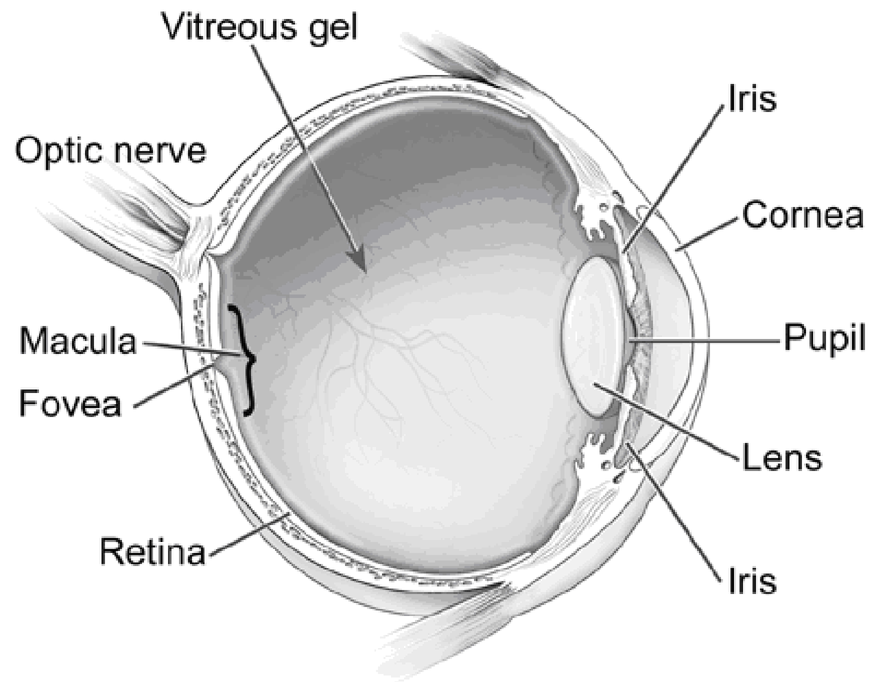


Figure 1.

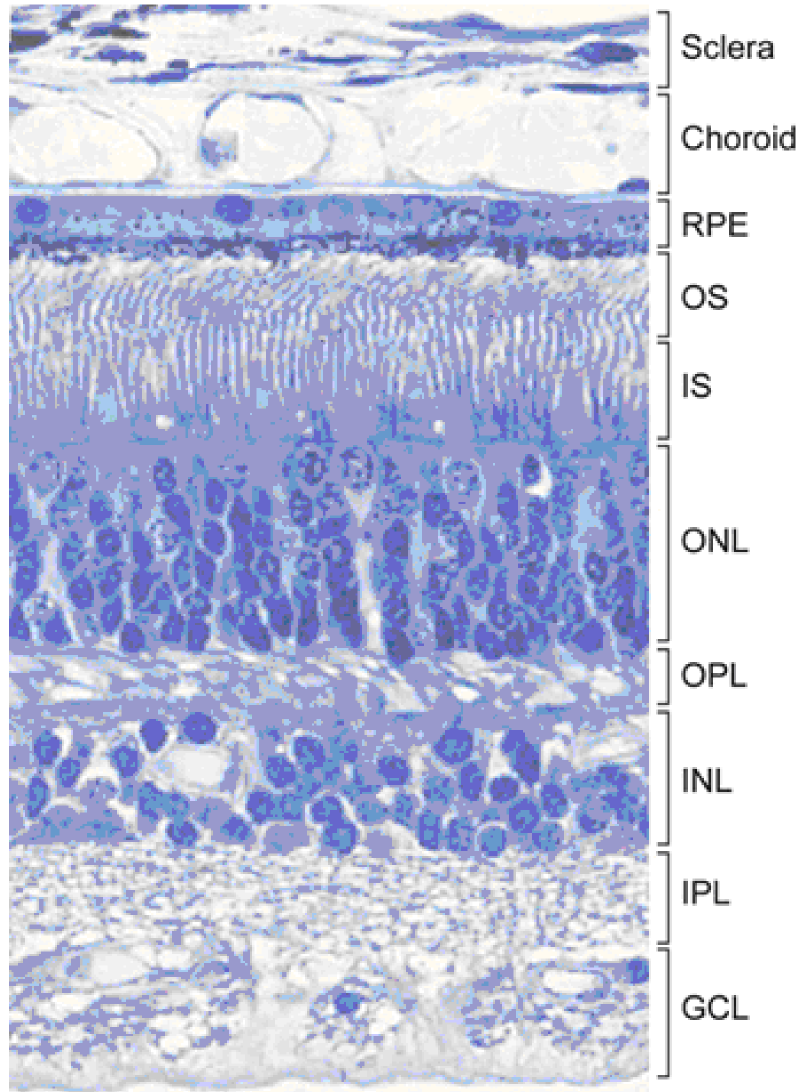
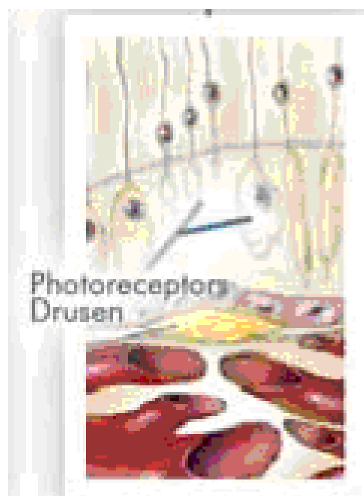


Figure 2.

A. **B.**



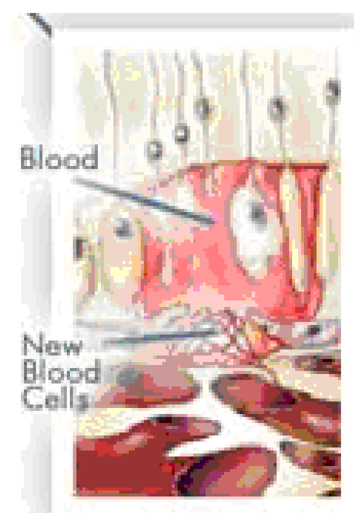


Figure 3.

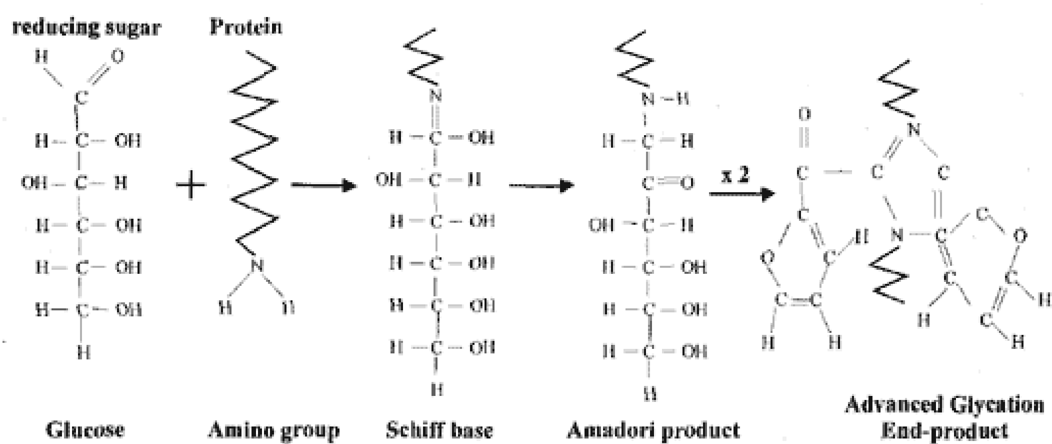


Figure 4.

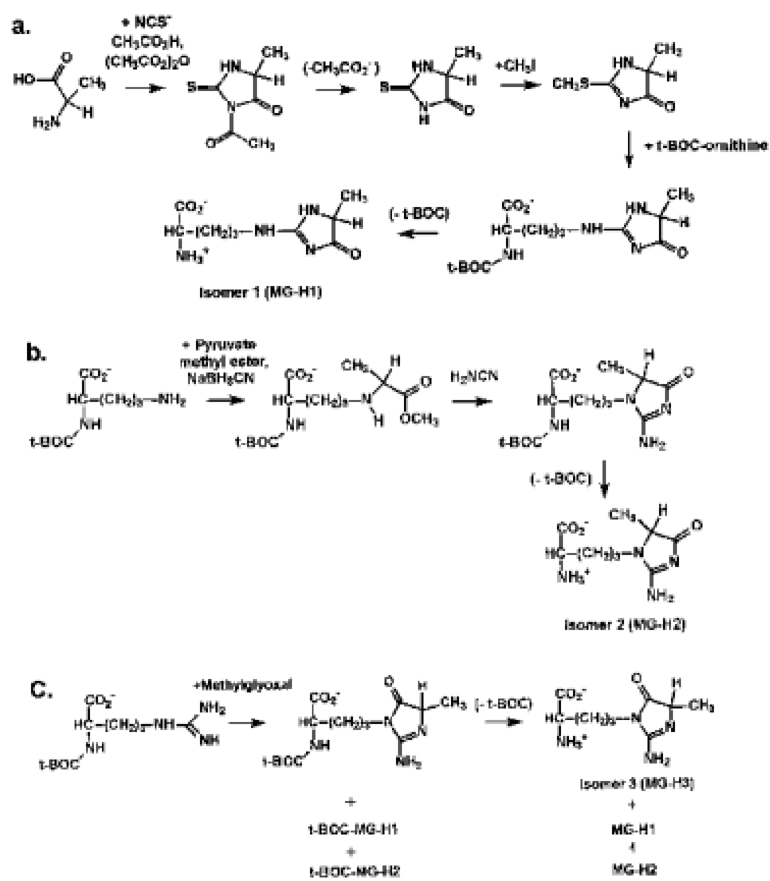


Figure 5.

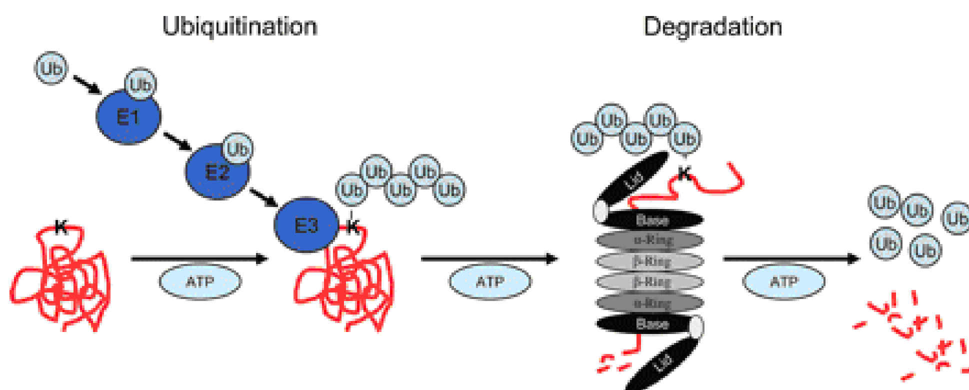


Figure 6.

Chapter 3. Natural History of AMD-Like Lesions in Mice Fed High or Low Glycemic Index Diets

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Word Count: 4,265

Funding: This research was funded by USDA 1950-510000-060-01A, Johnson and Johnson Focused Giving, and the American Health Assistance Foundation, NIH 13250, NIH 21212, NEI P30 EY12576 (UCD), EY14004, EY019904, Thome Foundation Award, Robert Bond Welch Professorship.

3.1 Abstract

Purpose: Epidemiologic data indicate that people who consume low glycemic index (**GI**) diets are at a reduced risk for the onset and progress of age-related macular degeneration (AMD). We sought corroboration of this observation in an animal model. **Methods**: Five and 16-month-old C57BL/6 mice were fed high or low GI diets until they were 17- and 23.5-months of age, respectively. AMD-like lesions were evaluated by transmission electron microscopy and advanced glycation end products (AGEs) were evaluated by immunohistochemistry. **Results**: AMD-like lesions including basal laminar deposits, loss of basal infoldings, and vacuoles in the retinal pigment epithelium were more prevalent in the 23.5- than in the 17-month-old mice. Within each age group, consumption of a high GI diet increased risk for AMD-like lesions, as well as risk for photoreceptor abnormalities and accumulation of AGEs. **Conclusion**: Consuming high GI diets accelerates the appearance of AMD-like lesions in mice, perhaps by increasing deposition of toxic AGEs in the retina. The data support the hypothesis that consuming lower GI diets, or simulation of their effects with nutraceuticals or drugs, may protect against AMD. The high GI-fed C57BL/6 mouse is a new model of AMD-like lesions that mimics the early stages of disease and may be useful for drug discovery.

3.2 Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly.[1] Available treatments for this devastating disease are

limited, targeting only advanced neovascular AMD. Only 10% of AMD is the neovascular form.[2-10] The remaining 90% of cases (approximately 7 million patients in the United States) are diagnosed with non-neovascular AMD, conditions for which treatment options are limited, and disease progress is inexorable.[10] The best strategy to alleviate the personal and public financial burden of this disease would be to prevent the onset and progression of AMD pathology.[9, 11-15] It has been estimated that slowing the progression rate to late-stage AMD by 30% would prevent AMD-related blindness by 50%.[16]

The preventive potential of various nutrients on retinal health has been explored in many observational studies and an intervention trial, the Age-Related Eye Disease Study (AREDS).[17-21] AREDS found that an antioxidant cocktail of vitamins C and E, beta-carotene, zinc, and copper reduced progression from intermediate to late-stage AMD. Unfortunately it had no effect on preventing early AMD.[22] Recent analyses of data from the AREDS, Nutrition and Vision Project sub-study of the Nurses' Health Study, and the Blue Mountains Eye Study revealed that the onset of early AMD (as well as progression through the stages of AMD) can be delayed through modulation of dietary carbohydrates. Specifically, it was observed that those who consumed diets containing carbohydrates of a high glycemic index (GI) were at an increased risk for AMD onset and progression, compared to those who consumed diets of a low GI. Importantly, this effect was independent of other nutrients that are thought to modulate risk for AMD.[23-28]

The GI of a food quantifies the rise in blood glucose following consumption of 50 grams of carbohydrate from that particular food relative to the rise in glucose levels following consumption of 50 grams of carbohydrate from a standard food (glucose, white bread).[29] Foods with a high GI induce a larger increase in blood glucose levels than foods with a low GI.

Consumption of low GI diets has also been associated with reduced risk for a number of other chronic diseases, such as type II diabetes, cardiovascular disease, and kidney disease.[25, 27, 30-38] By controlling spikes in blood sugar, low GI diets also result in lower levels of serum advanced glycation end products (AGEs), defined as proteins that are non-enzymatically modified by glucose or its metabolites.[39, 40] Increased levels of AGEs have been observed under conditions of oxidative stress and inflammation, as well as in several chronic diseases including AMD.[39, 41-70] However, there is a paucity of published information about relationships between tissue levels of AGEs and dietary GI.[71, 72]

A large number of mouse models of AMD have been developed. Many are transgenic models that exert acute, severe stresses on the retina.[73-101] We used a non-transgenic model and fed mice a high or low GI diet to determine if chronic intake of diets of different GI affects rates of appearance of AMD-like lesions and to begin to obtain insights into the pathophysiology of these relationships.[92] The lesions included accumulation of basal laminar deposits (BLDs), accumulation of BLD-associated membranous debris, loss of basal infoldings, vacuolization of the retinal pigment epithelium (RPE), loss of melanin, accumulation of outer collagenous layer deposits, accumulation of lipofuscin,

thickening of Bruch's membrane (BrM), disorganization of photoreceptor outer segments, thinning of outer photoreceptor nuclear and inner nuclear layers, and retinal accumulation of AGEs.

3.3 Methods

Ethics statement

This study was carried out and approved under the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University IACUC protocols, in accordance with the Animal Welfare Act provisions and the ARVO Statement for Use of Animals in Ophthalmic and Vision Research, and with all other animal welfare guidelines such as the NIH Guide for the Care and Use of Laboratory Animals.

Animals

Five- and 16-month-old male C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, MA). Ten 5-month-old mice were fed a high GI diet until 17 months of age (hereafter referred to as the 17-month-old high GI group) (**Table 1**). Another ten 5-month-old mice were fed a low GI diet (hereafter referred to as the 17-month-old low GI group) until 17 months of age (**Table 1**). The only difference between the high and low GI diets was the distribution of starch. The high GI diet starch was composed of 100% amylopectin, while the low GI diet starch was composed of 30% amylopectin/70% amylose. These diets were isocaloric and of identical macronutrient distribution (65% carbohydrate, 21% protein, 14% fat). An additional five 5-month-old mice served as controls

and were fed a high GI diet without hydroquinone (HQ) until 17 months of age. All 17-month-old mice were fed a high or low GI diet for 46 weeks prior to sacrifice.

We fed ten 16-month-old mice a high GI diet (hereafter referred to as the 23.5-month-old high GI group), and we fed an additional ten 16-month-old mice a low GI diet (hereafter referred to as the 23.5-month-old low GI group) until 23.5 months of age. These mice were fed their specific GI diet for 26 weeks prior to sacrifice.

In both age groups, the mice were pair-fed to ensure equal consumption between diet groups. All of the diets used in this study were formulated by Bio-Serv (Frenchtown, NJ). National Starch (Bridgewater, NJ) generously donated Amioca starch (100% amylopectin) for incorporation into the high GI diet, and Hylon VII starch (30% amylopectin/70% amylose) for incorporation into the low GI diet.

All mice were fasted 6 hours prior to being euthanized with carbon dioxide, and sacrificed by cervical dislocation. Eyes were enucleated for either transmission electron microscopy analysis or light microscopy analysis.

Transmission Electron Microscopy Analysis

After the mice were sacrificed, eyes were marked at the superior-most point using a cauterizing pen. To provide orientation for future analyses, eyes were enucleated, and at the cauterization mark either a suture was inserted or a cut was made in the sclera. For electron microscopy, the eyes were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer. Eyes were then post-

fixed in 1% osmium tetroxide buffered by 0.1M sodium cacodylate before dehydration with ethanol and embedding in epoxy resin. Proper orientation of the eyes was first confirmed by light microscopy analysis of thick sections. Ultrathin sections were then cut along the longitudinal axis from the central 2x2 mm area of the retina, 1 mm temporal to the optic nerve. Sections were stained with uranyl acetate and lead citrate and examined with a Philips CM-10 or Philips CM-120 electron microscope (Eindhoven, Netherlands). In the 17-month-old cohort, an average of 16 images were evaluated from each of 4 mice in the high GI, 4 mice in the low GI, and 3 mice in the control (non HQ) group. In the 23.5-month-old cohort, an average of 30 images were evaluated from each of the 3 mice in the high GI and 4 mice in the low GI group.

Grading Scheme

Retina lesions were evaluated according to previously published grading schemes[102, 103] with modifications to include age-related lesions such as BLD-associated membranous debris, RPE cytoplasmic vacuoles, loss of basal infoldings, melanin, and lipofuscin (**Table 2**). In each image, BrM thickness was determined by averaging the thickness at the thickest and thinnest points in an image. If the average BrM thickness was > 0.4 μm (the approximate thickness of BrM in a young, healthy mouse [104]), that image was scored as having a thickened BrM (score of 1). If the average BrM thickness in each image was $<$ 0.4 μm, that image was given a score of 0. The frequency score for each mouse was the sum of those images with a score of 1 divided by the total number of

images analyzed in that mouse. Mouse frequency scores were then averaged to find the frequency score for the entire diet or age group.

The frequency scores for other lesions were determined by counting the number of each lesion per μm in each image. In each mouse, a frequency score for a particular lesion was determined by averaging all of the frequency scores for a particular lesion from the individual images from that mouse. Mouse frequency scores were then averaged to determine the frequency score for the entire diet or age group. In addition, some characteristics were also graded on the severity of their appearance (scoring 0-3) (**Table 2**). In each mouse, a severity score for a particular lesion was determined by averaging all of the severity scores for a particular lesion from the individual images from that mouse. Mouse severity scores were then averaged to determine the severity score for the entire diet or age group. Specific measurement criteria are described in **Table 2**.

Student's t test was used to compare the frequencies of BLDs, loss of basal infoldings, cytoplasmic vacuoles, melanin, outer collagenous layer deposits, and lipofuscin between diet and age groups after the data were log-transformed. Wilcoxon's Mann Whitney U test was used to compare the frequencies of BLD-associated membranous debris and a thickened BrM between diet and age groups. Severity scores for BLDs, loss of basal infoldings, cytoplasmic vacuoles, outer collagenous layer deposits, and BrM were also compared between diet and age groups using Wilcoxon's Mann Whitney U test. All statistical analyses were carried out using SAS 9.2 (Cary, NC).

Immunohistochemistry

Eyes were isolated and their orientation preserved as described above. Following fixation in 4% paraformaldehyde and removing the lens, eye cups were embedded in paraffin and sectioned (5 μm thickness) from the central area of the retina, 1 mm temporal to the optic nerve along the longitudinal axis. Following deparaffinization with xylene and antigen retrieval with citrate buffer, sections were blocked with 5% goat serum (Jackson Immunoresearch Inc, West Grove, PA) in 1% BSA/TBS for 2 hours at room temperature.[105-107] Sections were then incubated with 0.13 mg/ml Affinpure fab fragment goat-anti-mouse (Jackson Immunoresearch Inc, West Grove, PA) to block endogenous mouse antibody. This was followed by incubation with avidin and biotin from Vectastain ABC Kit (Standard) (Vector Laboratories, Burlingame, CA) to block endogenous avidin and biotin. Sections were then incubated overnight at 4°C with 16.3 μg/ml α-MG-H1 (generously provided by M. Brownlee). After several washes, the sections were incubated with 1.7 μg/ml biotin-SP-conjugated goat-anti-mouse antibody (Jackson Immunoresearch Inc, West Grove, PA) for 30 minutes, and washed and incubated with 1:500 dilution of streptavidin-alkaline phosphatase (Vector Laboratories, Burlingame, CA) for 30 minutes. Antibody deposition was visualized with BCIP/NBT Alkaline Phosphatase Substrate kit (Vector Laboratories, Burlingame, CA) by following the manufacturer's instructions. For visualization of antigen present in the retinal pigment epithelial cells, sections were bleached after immunolabeling with 0.05% potassium permanganate (≥99%, Sigma, St. Louis, MO) for 25 minutes, followed by incubation with 35%

peracetic acid (FMC, Philadelphia, PA) for 25 minutes as per a modification of Bhutto et al.[108] Sections were then mounted with VectaMount AQ (Vector Laboratories, Burlingame, CA). Images were captured using the DP70 Digital Microscope Camera (Olympus, Center Valley, PA).

Sections were also stained with hematoxylin and eosin to assess the outer and inner nuclear layer thickness of mice fed a high GI (n=3) or low GI (n=3) diet. For each section, the number of rows of inner and outer nuclei was counted from three different regions of the retina: central, superior, and inferior. For each nuclear layer, these three counts were averaged to produce a single row count for each tissue section. The thickness of each nuclear layer for each mouse was determined by averaging the row counts from each tissue section from that mouse. The thickness of each nuclear layer in the entire diet or age group was determined by averaging the row counts from all of the mice in that group. Differences between groups were compared using Student's t test (SAS 9.2, Cary, NC).

3.4 Results

3.4.1 Frequency and Severity of AMD-Like Lesions in 17- and 23.5-Month-Old Mice of the Same Diet Group

To confirm that animals fed these experimental diets showed anticipated advancement of AMD-like lesions upon aging, we evaluated the frequency and severity of AMD-like lesions in 17- and 23.5-month-old mice in each diet group. Deposition on BrM of electron dense BLDs, especially those of a fibrillar or

collagenous composition (FD), is associated with aging and is also a precursor of AMD-like lesions.[109] BLDs were observed more frequently ($p < 0.05$) (henceforth, $p < 0.05$ was considered statistically significant, and $p < 0.1$ was considered marginally significant) and appeared to be of greater severity (as indicated by FD) in the 23.5-month-old mice than in the 17-month-old mice that were fed a high GI diet (**Figure 1B vs. 1A, Figure 2A, 2B**). BLDs were also more frequent in the 23.5- than in the 17-month-old mice fed a low GI diet ($p < 0.05$) (**Figure 2A**).

BLD-associated membranous debris are a feature associated with retinal disease as well as aging and are thought to precede the formation of basal linear deposits.[110, 111] BLD-associated membranous debris were more prevalent in the 23.5-month-old mice than in the 17-month-old mice fed the high GI diet ($p < 0.05$), and the same relationship was observed for the low-GI fed animals (**"MD" in Figure 1B, Figure 2C**). Similarly, RPE cytoplasmic vacuoles were more prevalent in the 23.5-month-old mice than in the 17-month-old mice that consumed the high GI and low GI diets ($p < 0.05$ in both diet groups) (**Figure 2D**).

BLDs often occupy space that was originally populated by basal infoldings. Consistent with the age-related increase in BLDs, the 23.5-month-old mice from both diet groups demonstrated greater severity of loss of basal infoldings than the 17-month-old mice ($p < 0.05$ and $p < 0.1$ for high GI and low GI groups, respectively) (**"+"** indicates the space where basal infoldings, if present, would be found in retina from younger mice, in **Figure 1B vs. 1A,**

Figure 2E). Another commonly used metric of retinal aging and disease is BrM thickness. BrM tended to be thicker in the 23.5-month-old mice compared to the 17-month-old mice that were fed the high GI diet, but variability within each age group precluded this difference from reaching statistical significance (**knobbed lines in Figure 1B vs. 1A**). Combined, these data clearly indicate that mice fed either diet show increased risk for many AMD-like lesions upon aging (**Figure 2A-E**).

Prior data indicate that melanin levels decrease upon aging, but melanin levels had not been measured previously in mice fed these diets.[112] We found an unexpected increase in the frequency of melanin pigment granules ($p < 0.05$) in the 23.5-month-old mice compared to the 17-month-old mice fed the high GI diet (**Figure 2F**). The same relationship, albeit muted, was also observed in the low GI-fed mice. Among the low GI-fed mice, there were also less severe (smaller) outer collagenous layer deposits ($p < 0.05$) and fewer lipofuscin granules ($p < 0.1$) in the 23.5-month-old mice relative to the 17-month-old mice (**Figure 2G, 2H**).

3.4.2 Frequency and Severity of AMD-Like Lesions in Age-Matched Mice Fed a High or Low GI Diet

We also compared the effects of dietary GI on the frequency and severity of retinal lesions in age-matched mice. In most cases, consuming the lower GI diet reduced or delayed the appearance of the AMD-like lesion. This was particularly obvious in the older animals. Thus, the frequency and severity of

BLDs was lower in 23.5-month-old mice that were fed the low GI diet compared to age-matched mice fed the high GI diet ($p < 0.05$) (**Figure 1D vs. 1B, Figure 2A, 2B**). Similar differences were observed for frequency of BLD-associated membranous debris, frequency of cytoplasmic vacuoles, severity of loss of basal infoldings, frequency of melanin, severity of outer collagenous layer deposits and frequency of lipofuscin deposition in the 23.5-month-old mice (**Figure 2C-H**). Some of these diet-related differences were also observed in the younger animals, including fewer BLDs, BLD-associated membranous debris and cytoplasmic vacuoles ($p < 0.05$) in the low GI-fed mice (**Figure 1C vs. 1A, Figure 2A, 2C, 2D**).

Overall, the differences in frequency and severity of AMD-like lesions appear to be greatest between low-GI fed younger animals and high-GI fed older animals. The most robust differences between 17-month-old low GI-fed mice and 23.5-month-old high GI-fed mice were observed in frequency of BLDs ($p < 0.001$), frequency of BLD-associated membranous debris ($p < 0.05$), frequency of cytoplasmic vacuoles ($p = 0.01$), severity of loss of basal infoldings ($p < 0.05$) and frequency of melanin ($p = 0.01$) (**Figure 2A, 2C-F**). For each of these lesions, the frequency or severity was greater in the high GI-fed 23.5-month-old mice, suggesting that older age and higher dietary GI accelerate the retinal changes that precede AMD.

3.4.3 Effects of Dietary GI on AGE Accumulation

Analyses of tissues from 11-month-old 129SvPas mice fed diets of differing GI for 10 months indicated that mice fed a high GI diet accumulated more MG-H1-modified proteins (hereafter called MG-H1) in their retinas compared to mice fed a low GI diet (Uchiki et al, 2011). MG-H1, one of the most common AGEs, is formed upon the reaction of methylglyoxal, a glucose metabolite, with protein.[113] If dietary glycemia, and therefore MG-H1 accumulation, were causally related to lesions, we would expect more severe lesions and MG-H1 accumulation in high GI-fed mice. To corroborate the association between consumption of a high GI diet and retinal MG-H1 accumulation in the present study, mouse retinas were analyzed immunohistochemically. RPE of 17-month-old mice fed the high GI diet had higher levels of MG-H1 than mice that consumed the low GI diet, as indicated in bleached (pink arrows in **Figure 3B vs. 3J**) and unbleached sections (pink arrows in **Figure 3D vs. 3L**). Increased deposition of MG-H1 in high GI rather than low GI-fed mouse retinas was also seen in layers of the inner retina, including the outer (yellow arrows) and inner (white arrows) nuclear layers, inner plexiform layer (orange arrows), and ganglion cell layer (black arrows) (**Figure 3A vs. 3I and 3C vs. 3K**). The observed AGE accumulation in retinal layers anterior to the RPE suggested that the high GI diet increased glycative stress throughout the retina, and prompted us to examine the effects of aging and dietary GI on retinal lesions interior to the RPE.

3.4.4 Damage to Photoreceptors and Inner Nuclei in 17- and 23.5-Month-Old Mice Fed High or Low GI Diets

In 17- and 23.5-month-old mice fed high or low GI diets, we examined the impact of aging and dietary GI on the integrity of photoreceptors that overlie the RPE. The prevalence of photoreceptor damage was low in all groups. However, more frequent focal photoreceptor outer segment vacuolization and disorganization was observed in high rather than low GI-fed mice (yellow arrows in **Figure 4A vs. 4B, Figure 4C vs. 4D**). We did not observe age-related differences.

In addition to outer segment damage, we also analyzed the effects of age and GI on the number of rows of photoreceptor outer nuclei, since thinning of this layer has been observed in models of AMD.[97] Aging was associated with a decrease in thickness of photoreceptor outer nuclear layers in both diet groups (**Table 3**, $p < 0.01$ and $p = 0.06$ for low and high GI-fed mice, respectively). Although suggestive, the difference in outer nuclear layer thickness between diet groups did not reach statistical significance (**Table 3**).

Analysis of the inner nuclear layer revealed that older age was associated with thinning of this layer in animals of the same diet group ($p < 0.01$ for both diet groups) (**Figure 5A vs. 5B and Figure 5C vs. 5D**) (**Table 4**). There was also a greater preservation of inner nuclear layers in the 23.5-month-old animals that consumed the low GI diet compared to age-matched mice that consumed the high GI diet ($p = 0.07$) (**Figure 5D vs. 5B**) (**Table 4**). In comparison, at 17 months of age, GI of the diet had less effect on the number of rows of inner

nuclei (**Figure 5A vs. 5C**) (**Table 4**). These data suggest that the stresses that are associated with thinning of the inner nuclear layer are prominent later in life and are related to dietary glycemia.

3.4.5 Comparison Between HQ and a High GI Diet as Retinal Stressors

The mouse model used in this study was originally designed to investigate the effects of cigarette smoke on AMD.[92] Therefore, HQ, a component of cigarette smoke, was included in the diets of both the high and low GI groups.[92] In order to evaluate the stress due to dietary HQ versus that of a high GI diet alone, we compared the retinal integrity between 17-month-old mice fed a high GI diet with HQ to those fed a high GI diet without HQ. Surprisingly, the singular insult of consuming the high GI diet resulted in higher levels of some AMD-like lesions than were observed when HQ was included in the diet. These include higher frequency ($p < 0.05$) and severity ($p < 0.1$) of BLDs (**Figure 6A vs. 6B, 6C, 6D**). Measures of other AMD-like lesions such as the frequency of BLD-associated membranous debris, severity of loss of basal infoldings, BrM thickness, frequency of lipofuscin, frequency of cytoplasmic vacuoles and the frequency and severity of outer collagenous layer deposits were indistinguishable between 17-month-old mice fed a high GI diet with HQ and age-matched mice fed a high GI diet without HQ (data not shown). Just as the inclusion of HQ into the diet did not exacerbate retinal stress, inclusion of HQ did not significantly increase metabolic stress as indicated by body weight, fasting glucose, glucose

tolerance, insulin tolerance, and glycated hemoglobin levels (**Supplemental Methods**, **Supplementary Figure S1**).

3.5 Discussion

In this work, we established a relationship between age, AMD-like lesions, and dietary GI in a murine model. The model faithfully recapitulates human epidemiologic data showing that aging is associated with more advanced lesions and that consuming low GI foods is associated with lower risk for onset and progress of these lesions. We also corroborated prior mechanistic findings by demonstrating tissue accumulation of AGEs in mice fed a higher GI diet and relating that to AGE accumulation in regions of age- and diet-associated pathology.

The absence of a macula limits the capacity of the rodent retina to completely model human AMD, so researchers using mouse models rely on evaluations of AMD-like lesions to determine associations between various treatments and risk for early stages of AMD.[89, 92, 97, 109, 111, 114-118] We found that upon aging, mice fed either a high or low GI diet showed increased retinal lesions such as accumulation of BLDs and cytoplasmic vacuoles, loss of basal infoldings, and loss of outer and inner nuclear layers, confirming previous reports of the age-associated nature of these lesions (**Figures 1-2**).[109, 115, 116, 119, 120] Importantly, we noted that in general, there were more robust age-related differences in lesion frequency and severity in high GI-fed mice than in low GI-fed

mice, indicating that consumption of a low GI diet attenuates and may delay AMD-like lesions.

The differences in frequency and severity of AMD-like lesions between diet groups of age-matched mice may be due to a difference in body weight between diet groups as well as the difference in dietary starches. In both 17- and 23.5-month old mice, the high GI-fed mice were heavier than the low GI-fed mice (data not shown). Although correlation analysis did not indicate that body weight was related to the appearance of AMD-like lesions, the difference in body weight could have influenced systemic stress levels. Another factor which may account for the difference in AMD-like lesions between diet groups is the ratio of amylopectin/amylose, which is higher in the higher GI diet. Amylopectin is digested at a faster rate than amylose, resulting in an increased flux of glucose and methylglyoxal into the retina.[121] Glycative stress in the retina is demonstrated by increased levels of MG-H1 in the RPE and photoreceptors (**Figure 3**).[122-125] Previously, it has been shown that glycative stress from increased glucose catabolism increases risk for diabetes and cardiovascular disease in humans. [126, 127] Our data suggest that this stress may impact the retina as well, creating an environment predisposed to the accumulation of lesions, as shown in **Figures 1, 2, 4, 5.** A plausible link between dietary GI, retinal stress, and AMD may be found in deposition of AGEs, the known cytotoxicity of AGEs, and the recently discovered impairment of protein editing caused by glycative stress in the retinas of mice consuming high GI diets (Uchiki et al, 2011).

The retinal lesions that were accelerated in the high GI-fed mice have also been observed in diabetic rodents, suggesting that it may be possible to use these data to gain insight into nutritional amelioration of diabetic retinopathy. These lesions include vacuolization of the RPE, disorganization of photoreceptor outer segments, decreased thickness of the inner nuclear layer, and accumulation of AGEs in the inner retina.[128-133] AGEs directly contribute to the vascular compromises of diabetic retinopathy by increasing levels of vascular endothelial growth factor (VEGF).[134-141] Thus, it seems that consuming lower GI diets should reduce AGEs and the associated risk for progress of diabetic retinopathy as well as AMD.

Implementation of the high GI diet, with or without HQ, allowed us to evaluate the role of HQ in retinal aging and the etiology of AMD-like lesions. Higher levels of lesions were not observed in animals that consumed a high GI diet with, rather than without HQ, suggesting that HQ is not necessary to elicit these AMD-like lesions in animals under these conditions.

This study shows that age is associated with AMD-like lesions in the murine retina and that consuming high GI diets augments this pathology. Overall, the differences in AMD-like lesions appear to be greatest between low GI-fed younger animals and high GI-fed older animals. The C57BL/6 mouse fed a high GI diet mimics the processes of retinal aging and development of AMD-like lesions in humans. As such, it provides a new excellent platform to study effects of modulators (drugs, nutraceuticals) on aging and risk for early AMD.

These data show that the model is responsive to environmental influences such as nutrition and will also be useful for studies of mechanisms of disease initiation.

3.6 References

1. Gehrs KM, Anderson DH, Johnson LV, Hageman GS. Age-related macular degeneration--emerging pathogenetic and therapeutic concepts. *Ann Med* 2006;38:450-471.

2. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. *Ophthalmology* 2009;116:57-65 e55.

3. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med* 2006;355:1419-1431.

4. Gragoudas ES, Adamis AP, Cunningham ET, Jr., Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med* 2004;351:2805-2816.

5. Friberg TR, Tolentino M, Weber P, Patel S, Campbell S, Goldbaum M. Pegaptanib sodium as maintenance therapy in neovascular age-related macular degeneration: the LEVEL study. *Br J Ophthalmol* 2010;94:1611-1617.

6. Forte R, Cennamo G, Finelli M, et al. Intravitreal triamcinolone, bevacizumab and pegaptanib for occult choroidal neovascularization. *Acta Ophthalmol* 2010;88:e305-310.

7. Tufail A, Patel PJ, Egan C, et al. Bevacizumab for neovascular age related macular degeneration (ABC Trial): multicentre randomised double masked study. *Bmj* 2010;340:c2459.

8. Biarnes M, Mones J, Alonso J, Arias L. Update on Geographic Atrophy in Age-Related Macular Degeneration. *Optom Vis Sci* 2011.

9. Frick KD, Gower EW, Kempen JH, Wolff JL. Economic impact of visual impairment and blindness in the United States. *Arch Ophthalmol* 2007;125:544-550.

10. Klein R, Chou CF, Klein BE, Zhang X, Meuer SM, Saaddine JB. Prevalence of age-related macular degeneration in the US population. *Arch Ophthalmol* 2011;129:75-80.

11. Ivers RQ, Norton R, Cumming RG, Butler M, Campbell AJ. Visual impairment and risk of hip fracture. *Am J Epidemiol* 2000;152:633-639.

12. Desrosiers J, Wanet-Defalque MC, Temisjian K, et al. Participation in daily activities and social roles of older adults with visual impairment. *Disabil Rehabil* 2009;31:1227-1234.

13. Dargent-Molina P, Favier F, Grandjean H, et al. Fall-related factors and risk of hip fracture: the EPIDOS prospective study. *Lancet* 1996;348:145-149.

14. Williams RA, Brody BL, Thomas RG, Kaplan RM, Brown SI. The psychosocial impact of macular degeneration. *Arch Ophthalmol* 1998;116:514-520.

15. Wood J, Lacherez P, Black A, Cole M, Boon M, Kerr G. Risk of falls, injurious falls, and other injuries resulting from visual impairment among older adults with Age-related Macular Degeneration. *Invest Ophthalmol Vis Sci* 2011.

16. The Complications of Age-Related Macular Degeneration Prevention Trial (CAPT): rationale, design and methodology. *Clin Trials* 2004;1:91-107.

17. Chiu CJ, Taylor A. Nutritional antioxidants and age-related cataract and maculopathy. *Exp Eye Res* 2007;84:229-245.

18. Ma L, Lin XM. Effects of lutein and zeaxanthin on aspects of eye health. *J Sci Food Agric* 2010;90:2-12.

19. Coleman H, Chew E. Nutritional supplementation in age-related macular degeneration. *Curr Opin Ophthalmol* 2007;18:220-223.

20. Weikel K, Taylor, A. Nutritional Modulation of Cataract and Age-Related Macular Degeneration. *Duanes Ophthalmology* 2011;in press.

21. The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. *Control Clin Trials* 1999;20:573-600.

22. Chew EY, Lindblad AS, Clemons T. Summary results and recommendations from the age-related eye disease study. *Arch Ophthalmol* 2009;127:1678-1679.

23. Chiu CJ, Klein R, Milton RC, Gensler G, Taylor A. Diet, Supplement and Risk of Age-Related Macular Degeneration in the Age-Related Eye Disease Study. *Investigative Ophthalmology & Visual Science* 2008;49:597.

24. Chiu CJ, Milton RC, Klein R, Gensler G, Taylor A. Dietary carbohydrate and the progression of age-related macular degeneration: a prospective study from the Age-Related Eye Disease Study. *Am J Clin Nutr* 2007;86:1210-1218.

25. Chiu CJ, Milton RC, Gensler G, Taylor A. Association between dietary glycemic index and age-related macular degeneration in nondiabetic participants in the Age-Related Eye Disease Study. *Am J Clin Nutr* 2007;86:180-188.

26. Chiu CJ, Klein R, Milton RC, Gensler G, Taylor A. Does eating particular diets alter risk of age-related macular degeneration in users of the age-related eye disease study supplements? *Br J Ophthalmol* 2009;1241-1246.

27. Chiu CJ, Hubbard LD, Armstrong J, et al. Dietary glycemic index and carbohydrate in relation to early age-related macular degeneration. *Am J Clin Nutr* 2006;83:880-886.

28. Kaushik S, Wang JJ, Flood V, et al. Dietary glycemic index and the risk of age-related macular degeneration. *Am J Clin Nutr* 2008;88:1104-1110.

29. Jenkins DJ, Wolever TM, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 1981;34:362-366.

30. Ferland A, Brassard P, Lemieux S, et al. Impact of high-fat /low-carbohydrate, high-, low-glycaemic index or low-caloric meals on glucose regulation during aerobic exercise in Type 2 diabetes. *Diabet Med* 2009;26:589-595.

31. Jenkins DJ, Kendall CW, McKeown-Eyssen G, et al. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. *Jama* 2008;300:2742-2753.

32. McKeown NM, Meigs JB, Liu S, et al. Dietary carbohydrates and cardiovascular disease risk factors in the Framingham offspring cohort. *J Am Coll Nutr* 2009;28:150-158.

33. Liu S, Stampfer MJ, Hu FB, et al. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* 1999;70:412-419.

34. Riegersperger M, Sunder-Plassmann G. How to prevent progression to end stage renal disease. *J Ren Care* 2007;33:105-107.

35. Gopinath B, Harris DC, Flood VM, Burlutsky G, Brand-Miller J, Mitchell P. Carbohydrate nutrition is associated with the 5-year incidence of chronic kidney disease. *J Nutr* 2011;141:433-439.

36. Wolever TM, Jenkins DJ, Vuksan V, Jenkins AL, Wong GS, Josse RG. Beneficial effect of low-glycemic index diet in overweight NIDDM subjects. *Diabetes Care* 1992;15:562-564.

37. Chiu CJ, Milton RC, Klein R, Gensler G, Taylor A. Dietary Glycemic Index Is Related to Progression of Age-Related Macular Degeneration. *Investigative Ophthalmology & Visual Science* 2007;48:2101.

38. Sakurai M, Nakamura K, Miura K, et al. Dietary glycemic index and risk of type 2 diabetes mellitus in middle-aged Japanese men. *Metabolism* 2011.

39. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia* 2001;44:129-146.

40. Fu MX, Wells-Knecht KJ, Blackledge JA, Lyons TJ, Thorpe SR, Baynes JW. Glycation, glycoxidation, and cross-linking of collagen by glucose. Kinetics,

mechanisms, and inhibition of late stages of the Maillard reaction. *Diabetes* 1994;43:676-683.

41. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology* 2005;15:16R-28R.

42. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 2001;108:949-955.

43. Schmidt AM, Yan SD, Brett J, Mora R, Nowygrod R, Stern D. Regulation of human mononuclear phagocyte migration by cell surface-binding proteins for advanced glycation end products. *J Clin Invest* 1993;91:2155-2168.

44. Schmidt AM, Hori O, Chen JX, et al. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 1995;96:1395-1403.

45. Miyata T, Hori O, Zhang J, et al. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 1996;98:1088-1094.

46. Owen WF, Jr., Hou FF, Stuart RO, et al. Beta 2-microglobulin modified with advanced glycation end products modulates collagen synthesis by human fibroblasts. *Kidney Int* 1998;53:1365-1373.

47. Sakaguchi T, Yan SF, Yan SD, et al. Central role of RAGE-dependent neointimal expansion in arterial restenosis. *J Clin Invest* 2003;111:959-972.

48. Che W, Asahi M, Takahashi M, et al. Selective induction of heparin-binding epidermal growth factor-like growth factor by methylglyoxal and 3-deoxyglucosone in rat aortic smooth muscle cells. The involvement of reactive oxygen species formation and a possible implication for atherogenesis in diabetes. *J Biol Chem* 1997;272:18453-18459.

49. Makita Z, Yanagisawa K, Kuwajima S, Bucala R, Vlassara H, Koike T. The role of advanced glycosylation end-products in the pathogenesis of atherosclerosis. *Nephrol Dial Transplant* 1996;11 Suppl 5:31-33.

50. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. *Cardiovasc Res* 1998;37:586-600.

51. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* 1997;99:457-468.

52. Sasaki N, Fukatsu R, Tsuzuki K, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. *Am J Pathol* 1998;153:1149-1155.

53. Brownlee M. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 1992;15:1835-1843.

54. Sugiyama S, Miyata T, Ueda Y, et al. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. *J Am Soc Nephrol* 1998;9:1681-1688.

55. Kilhovd BK, Giardino I, Torjesen PA, et al. Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. *Metabolism* 2003;52:163-167.

56. Kilhovd BK, Juutilainen A, Lehto S, et al. Increased serum levels of methylglyoxal-derived hydroimidazolone-AGE are associated with increased cardiovascular disease mortality in nondiabetic women. *Atherosclerosis* 2009;205:590-594.

57. Fosmark DS, Berg JP, Jensen AB, et al. Increased retinopathy occurrence in type 1 diabetes patients with increased serum levels of the advanced glycation endproduct hydroimidazolone. *Acta Ophthalmol* 2009;87:498-500.

58. Han Y, Randell E, Vasdev S, et al. Plasma advanced glycation endproduct, methylglyoxal-derived hydroimidazolone is elevated in young, complication-free patients with Type 1 diabetes. *Clin Biochem* 2009;42:562-569.

59. Araki N, Ueno N, Chakrabarti B, Morino Y, Horiuchi S. Immunochemical evidence for the presence of advanced glycation end products in human lens proteins and its positive correlation with aging. *J Biol Chem* 1992;267:10211-10214.

60. Snow LM, Fugere NA, Thompson LV. Advanced glycation end-product accumulation and associated protein modification in type II skeletal muscle with aging. *J Gerontol A Biol Sci Med Sci* 2007;62:1204-1210.

61. Semba RD, Fink JC, Sun K, Windham BG, Ferrucci L. Serum carboxymethyl-lysine, a dominant advanced glycation end product, is associated with chronic kidney disease: the Baltimore longitudinal study of aging. *J Ren Nutr* 2010;20:74-81.

62. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. *J Am Geriatr Soc* 2009;57:1874-1880.

63. Howes K, Liu Y, Dunaief J, et al. Receptor for advanced glycation end products and age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2004;45:3713-3720.

64. Torreggiani M, Liu H, Wu J, et al. Advanced glycation end product receptor-1 transgenic mice are resistant to inflammation, oxidative stress, and post-injury intimal hyperplasia. *Am J Pathol* 2009;175:1722-1732.

65. Hofmann SM, Dong HJ, Li Z, et al. Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes* 2002;51:2082-2089.

66. Cai W, He JC, Zhu L, et al. Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. *Am J Pathol* 2008;173:327-336.

67. Vlassara H, Cai W, Goodman S, et al. Protection against Loss of Innate Defenses in Adulthood by Low Advanced Glycation End Products (AGE) Intake: Role of the Antiinflammatory AGE Receptor-1. *J Clin Endocrinol Metab* 2009.

68. Ma W, Lee SE, Guo J, et al. RAGE ligand upregulation of VEGF secretion in ARPE-19 cells. *Invest Ophthalmol Vis Sci* 2007;48:1355-1361.

69. Neumann A, Schinzel R, Palm D, Riederer P, Munch G. High molecular weight hyaluronic acid inhibits advanced glycation endproduct-induced NF-kappaB activation and cytokine expression. *FEBS Lett* 1999;453:283-287.

70. Sato T, Wu X, Shimogaito N, Takino J, Yamagishi S, Takeuchi M. Effects of high-AGE beverage on RAGE and VEGF expressions in the liver and kidneys. *Eur J Nutr* 2009;48:6-11.

71. Chiarelli F, de Martino M, Mezzetti A, et al. Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. *J Pediatr* 1999;134:486-491.

72. Kostolanska J, Jakus V, Barak L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2009;22:433-442.

73. Karan G, Lillo C, Yang Z, et al. Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant ELOVL4 transgenic mice: a model for macular degeneration. *Proc Natl Acad Sci U S A* 2005;102:4164-4169.

74. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med* 2003;9:1390-1397.

75. Rakoczy PE, Zhang D, Robertson T, et al. Progressive age-related changes similar to age-related macular degeneration in a transgenic mouse model. *Am J Pathol* 2002;161:1515-1524.

76. Dithmar S, Curcio CA, Le NA, Brown S, Grossniklaus HE. Ultrastructural changes in Bruch's membrane of apolipoprotein E-deficient mice. *Invest Ophthalmol Vis Sci* 2000;41:2035-2042.

77. Hadziahmetovic M, Dentchev T, Song Y, et al. Ceruloplasmin/hephaestin knockout mice model morphologic and molecular features of AMD. *Invest Ophthalmol Vis Sci* 2008;49:2728-2736.

78. Hu W, Jiang A, Liang J, et al. Expression of VLDLR in the retina and evolution of subretinal neovascularization in the knockout mouse model's retinal angiomatous proliferation. *Invest Ophthalmol Vis Sci* 2008;49:407-415.

79. Rakoczy EP, Yu MJ, Nusinowitz S, Chang B, Heckenlively JR. Mouse models of age-related macular degeneration. *Exp Eye Res* 2006;82:741-752.

80. Maeda A, Golczak M, Maeda T, Palczewski K. Limited roles of Rdh8, Rdh12, and Abca4 in all-trans-retinal clearance in mouse retina. *Invest Ophthalmol Vis Sci* 2009;50:5435-5443.

81. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* 1999;98:13-23.

82. Radu RA, Mata NL, Bagla A, Travis GH. Light exposure stimulates formation of A2E oxiranes in a mouse model of Stargardt's macular degeneration. *Proc Natl Acad Sci U S A* 2004;101:5928-5933.

83. Mata NL, Weng J, Travis GH. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc Natl Acad Sci U S A* 2000;97:7154-7159.

84. Hahn P, Qian Y, Dentchev T, et al. Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. *Proc Natl Acad Sci U S A* 2004;101:13850-13855.

85. Zhang D, Lai MC, Constable IJ, Rakoczy PE. A model for a blinding eye disease of the aged. *Biogerontology* 2002;3:61-66.

86. Heckenlively JR, Hawes NL, Friedlander M, et al. Mouse model of subretinal neovascularization with choroidal anastomosis. *Retina* 2003;23:518-522.

87. Espinosa-Heidmann DG, Sall J, Hernandez EP, Cousins SW. Basal laminar deposit formation in APO B100 transgenic mice: complex interactions between dietary fat, blue light, and vitamin E. *Invest Ophthalmol Vis Sci* 2004;45:260-266.

88. Callow MJ, Stoltzfus LJ, Lawn RM, Rubin EM. Expression of human apolipoprotein B and assembly of lipoprotein(a) in transgenic mice. *Proc Natl Acad Sci U S A* 1994;91:2130-2134.

89. Fujihara M, Bartels E, Nielsen LB, Handa JT. A human apoB100 transgenic mouse expresses human apoB100 in the RPE and develops features of early AMD. *Exp Eye Res* 2009;88:1115-1123.

90. Provost AC, Vede L, Bigot K, et al. Morphologic and electroretinographic phenotype of SR-BI knockout mice after a long-term atherogenic diet. *Invest Ophthalmol Vis Sci* 2009;50:3931-3942.

91. Cousins SW, Espinosa-Heidmann DG, Alexandridou A, Sall J, Dubovy S, Csaky K. The role of aging, high fat diet and blue light exposure in an experimental mouse model for basal laminar deposit formation. *Exp Eye Res* 2002;75:543-553.

92. Espinosa-Heidmann DG, Suner IJ, Catanuto P, Hernandez EP, Marin-Castano ME, Cousins SW. Cigarette Smoke-Related Oxidants and the Development of Sub-RPE Deposits in an Experimental Animal Model of Dry AMD. *Invest Ophthalmol Vis Sci* 2006;47:729-737.

93. Schwesinger C, Yee C, Rohan RM, et al. Intrachoroidal neovascularization in transgenic mice overexpressing vascular endothelial growth factor in the retinal pigment epithelium. *Am J Pathol* 2001;158:1161-1172.

94. Weber BH, Lin B, White K, et al. A mouse model for Sorsby fundus dystrophy. *Invest Ophthalmol Vis Sci* 2002;43:2732-2740.

95. Matsui H, Lin LR, Singh DP, Shinohara T, Reddy VN. Lens epithelium-derived growth factor: increased survival and decreased DNA breakage of human RPE cells induced by oxidative stress. *Invest Ophthalmol Vis Sci* 2001;42:2935-2941.

96. Frykman PK, Brown MS, Yamamoto T, Goldstein JL, Herz J. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 1995;92:8453-8457.

97. Justilien V, Pang JJ, Renganathan K, et al. SOD2 Knockdown Mouse Model of Early AMD. *Invest Ophthalmol Vis Sci* 2007;48:4407-4420.

98. Rudolf M, Winkler B, Aherrahou Z, Doehring LC, Kaczmarek P, Schmidt-Erfurth U. Increased expression of vascular endothelial growth factor associated with accumulation of lipids in Bruch's membrane of LDL receptor knockout mice. *Br J Ophthalmol* 2005;89:1627-1630.

99. Hollyfield JG, Perez VL, Salomon RG. A hapten generated from an oxidation fragment of docosahexaenoic acid is sufficient to initiate age-related macular degeneration. *Mol Neurobiol* 2010;41:290-298.

100. Ufret-Vincenty RL, Aredo B, Liu X, et al. Transgenic mice expressing variants of complement factor H develop AMD-like retinal findings. *Invest Ophthalmol Vis Sci* 2010;51:5878-5887.

101. Coffey PJ, Gias C, McDermott CJ, et al. Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction. *Proc Natl Acad Sci U S A* 2007;104:16651-16656.

102. Marin-Castano ME, Striker GE, Alcazar O, Catanuto P, Espinosa-Heidmann DG, Cousins SW. Repetitive Nonlethal Oxidant Injury to Retinal Pigment Epithelium Decreased Extracellular Matrix Turnover In Vitro and

Induced Sub-RPE Deposits In Vivo. *Invest Ophthalmol Vis Sci* 2006;47:4098-4112.

103. Ida H, Ishibashi K, Reiser K, Hjelmeland LM, Handa JT. Ultrastructural aging of the RPE-Bruch's membrane-choriocapillaris complex in the D-galactose-treated mouse. *Invest Ophthalmol Vis Sci* 2004;45:2348-2354.

104. Mishima H, Hasebe H. Some observations in the fine structure of age changes of the mouse retinal pigment epithelium. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 1978;209:1-9.

105. Takai H, Kato A, Ishiguro T, et al. Optimization of tissue processing for immunohistochemistry for the detection of human glypican-3. *Acta Histochem* 2010;112:240-250.

106. Ha SK, Choi C, Chae C. Development of an optimized protocol for the detection of classical swine fever virus in formalin-fixed, paraffin-embedded tissues by seminested reverse transcription-polymerase chain reaction and comparison with in situ hybridization. *Res Vet Sci* 2004;77:163-169.

107. Evke E, Minbay FZ, Temel SG, Kahveci Z. Immunohistochemical detection of p53 protein in basal cell skin cancer after microwave-assisted antigen retrieval. *J Mol Histol* 2009;40:13-21.

108. Bhutto IA, Kim SY, McLeod DS, et al. Localization of collagen XVIII and the endostatin portion of collagen XVIII in aged human control eyes and eyes with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2004;45:1544-1552.

109. Sarks SH, Arnold JJ, Killingsworth MC, Sarks JP. Early drusen formation in the normal and aging eye and their relation to age related maculopathy: a clinicopathological study. *Br J Ophthalmol* 1999;83:358-368.

110. Sarks S, Cherepanoff S, Killingsworth M, Sarks J. Relationship of Basal laminar deposit and membranous debris to the clinical presentation of early age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2007;48:968-977.

111. Marmorstein LY, McLaughlin PJ, Peachey NS, Sasaki T, Marmorstein AD. Formation and progression of sub-retinal pigment epithelium deposits in Efemp1 mutation knock-in mice: a model for the early pathogenic course of macular degeneration. *Hum Mol Genet* 2007;16:2423-2432.

112. Weiter JJ, Delori FC, Wing GL, Fitch KA. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci* 1986;27:145-152.

113. Rabbani N, Thornalley PJ. Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. *Amino Acids* 2010.

114. Tso MO. Pathogenetic factors of aging macular degeneration. *Ophthalmology* 1985;92:628-635.

115. Lewis H, Straatsma BR, Foos RY, Lightfoot DO. Reticular degeneration of the pigment epithelium. *Ophthalmology* 1985;92:1485-1495.

116. Sarks SH. Ageing and degeneration in the macular region: a clinicopathologic study. *Br J Ophthalmol* 1976;60:324-341.

117. Fujihara M, Nagai N, Sussan TE, Biswal S, Handa JT. Chronic cigarette smoke causes oxidative damage and apoptosis to retinal pigmented epithelial cells in mice. *PLoS ONE* 2008;3:e3119.

118. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA. An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. *Proc Natl Acad Sci U S A* 2009;106:18751-18756.

119. Tso MOM. Pathogenetic factors of aging macular degeneration. *Ophthalmology* 1985;92:628-635.

120. Picard E, Ranchon-Cole I, Jonet L, et al. Light-induced retinal degeneration correlates with changes in iron metabolism gene expression, ferritin level, and aging. *Invest Ophthalmol Vis Sci* 2011;52:1261-1274.

121. Brouwers O, Niessen PM, Haenen G, et al. Hyperglycaemia-induced impairment of endothelium-dependent vasorelaxation in rat mesenteric arteries is mediated by intracellular methylglyoxal levels in a pathway dependent on oxidative stress. *Diabetologia* 2010;53:989-1000.

122. Ader M, Bergman RN. Insulin sensitivity in the intact organism. *Baillieres Clin Endocrinol Metab* 1987;1:879-910.

123. Anderson GH, Cho CE, Akhavan T, Mollard RC, Luhovyy BL, Finocchiaro ET. Relation between estimates of cornstarch digestibility by the Englyst in vitro method and glycemic response, subjective appetite, and short-term food intake in young men. *Am J Clin Nutr* 2011;91:932-939.

124. So PW, Yu WS, Kuo YT, et al. Impact of resistant starch on body fat patterning and central appetite regulation. *PLoS One* 2007;2:e1309.

125. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr* 2005;82:559-567.

126. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet* 2002;359:2072-2077.

127. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *Jama* 2003;290:486-494.

128. Omri S, Behar-Cohen F, de Kozak Y, et al. Microglia/Macrophages Migrate through Retinal Epithelium Barrier by a Transcellular Route in Diabetic Retinopathy Role of PKCzeta in the Goto Kakizaki Rat Model. *Am J Pathol* 2011;179:942-953.

129. Tso MO, Cunha-Vaz JG, Shih CY, Jones CW. Clinicopathologic study of blood-retinal barrier in experimental diabetes mellitus. *Arch Ophthalmol* 1980;98:2032-2040.

130. Arnal E, Miranda M, Johnsen-Soriano S, et al. Beneficial effect of docosahexanoic acid and lutein on retinal structural, metabolic, and functional abnormalities in diabetic rats. *Curr Eye Res* 2009;34:928-938.

131. Sasaki M, Ozawa Y, Kurihara T, et al. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia* 2010;53:971-979.

132. Tang L, Zhang Y, Jiang Y, et al. Dietary wolfberry ameliorates retinal structure abnormalities in db/db mice at the early stage of diabetes. *Exp Biol Med (Maywood)* 2011.

133. Sohn EJ, Kim YS, Kim CS, Lee YM, Kim JS. KIOM-79 prevents apoptotic cell death and AGEs accumulation in retinas of diabetic db/db mice. *J Ethnopharmacol* 2009;121:171-174.

134. Lu M, Kuroki M, Amano S, et al. Advanced glycation end products increase retinal vascular endothelial growth factor expression. *J Clin Invest* 1998;101:1219-1224.

135. Stitt AW, Bhaduri T, McMullen CB, Gardiner TA, Archer DB. Advanced glycation end products induce blood-retinal barrier dysfunction in normoglycemic rats. *Mol Cell Biol Res Commun* 2000;3:380-388.

136. Mamputu JC, Renier G. Advanced glycation end products increase, through a protein kinase C-dependent pathway, vascular endothelial growth factor expression in retinal endothelial cells. Inhibitory effect of gliclazide. *J Diabetes Complications* 2002;16:284-293.

137. Murata T, Nakagawa K, Khalil A, Ishibashi T, Inomata H, Sueishi K. The relation between expression of vascular endothelial growth factor and breakdown of the blood-retinal barrier in diabetic rat retinas. *Lab Invest* 1996;74:819-825.

138. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 1988;318:1315-1321.

139. Vlassara H, Bucala R. Recent progress in advanced glycation and diabetic vascular disease: role of advanced glycation end product receptors. *Diabetes* 1996;45 Suppl 3:S65-66.

140. Yamagishi S, Nakamura K, Matsui T, et al. Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. *J Biol Chem* 2006;281:20213-20220.

141. Okamoto T, Yamagishi S, Inagaki Y, et al. Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. *Faseb J* 2002;16:1928-1930.

142. Bonilha VL. Age and disease-related structural changes in the retinal pigment epithelium. *Clin Ophthalmol* 2008;2:413-424.

143. Curcio CA, Millican CL. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch Ophthalmol* 1999;117:329-339.

144. Cherepanoff S, McMenamin P, Gillies MC, Kettle E, Sarks SH. Bruch's membrane and choroidal macrophages in early and advanced age-related macular degeneration. *Br J Ophthalmol* 2010;94:918-925.

145. Bonilha VL, Rayborn ME, Saotome I, McClatchey AI, Hollyfield JG. Microvilli defects in retinas of ezrin knockout mice. *Exp Eye Res* 2006;82:720-729.

146. Sundelin SP, Nilsson SE, Brunk UT. Lipofuscin-formation in cultured retinal pigment epithelial cells is related to their melanin content. *Free Radic Biol Med* 2001;30:74-81.

147. Rozanowski B, Cuenco J, Davies S, et al. The phototoxicity of aged human retinal melanosomes. *Photochem Photobiol* 2008;84:650-657.

148. Luhmann UF, Robbie S, Munro PM, et al. The drusenlike phenotype in aging Ccl2-knockout mice is caused by an accelerated accumulation of swollen autofluorescent subretinal macrophages. *Invest Ophthalmol Vis Sci* 2009;50:5934-5943.

149. Xu H, Chen M, Manivannan A, Lois N, Forrester JV. Age-dependent accumulation of lipofuscin in perivascular and subretinal microglia in experimental mice. *Aging Cell* 2008;7:58-68.

150. Tuo J, Bojanowski CM, Zhou M, et al. Murine ccl2/cx3cr1 deficiency results in retinal lesions mimicking human age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2007;48:3827-3836.

3.7 Figure Legends

Figure 1. AMD-like lesions are more advanced in C57BL/6 mice that are older and that consumed the high GI diet. Electron micrographs of (**A**) 17-month-old high GI-fed, (**B**) 23.5-month-old high GI-fed, (**C**) 17-month-old low GI-fed, and (**D**) 23.5-month-old low GI-fed mice are shown. Note larger more advanced BLDs, greater loss of BI, and vacuolization of cytoplasm in panel **B** vs. panels **A**, **C**, and **D**. BI = basal infolding; BLD = basal laminar deposit; BrM = Bruch's membrane (knobbed lines indicate approximate thickness of Bruch's Membrane); FD = fibrillar deposit; MD = BLD-associated membranous debris;

RPE = retinal pigment epithelium; V = vacuoles; + indicates a loss of basal infoldings. Bar = 1 μm.

Figure 2. Semi-quantitative comparison of AMD-like features between 17- and 23.5-month-old mice fed high or low GI diets. Mean values for 17-month-old high GI (blue bars), 17-month-old low GI (red bars), 23.5-month-old high GI (green bars), and 23.5-month-old low GI (purple bars) groups are shown for frequency of basal laminar deposits (**A**), severity of basal laminar deposits (**B**), frequency of basal laminar deposit-associated membranous debris (**C**), frequency of cytoplasmic vacuoles (**D**), severity of loss of basal infoldings (**E**), frequency of melanin (**F**), severity of outer collagenous layer deposits (**G**), and frequency of lipofuscin (**H**). Error bars represent SEM. ** indicates $p < 0.05$ and # indicates $p < 0.1$. In order to keep the figure clear, p values for comparison of frequency and severity of lesions between 17-month-old low GI-fed and 23.5-month-old high GI-fed mice are not shown. These statistics are indicated in the text.

Figure 3. MG-H1-modified protein accumulates in the retinas of mice fed a high GI diet. Retinas from 17-month-old mice fed high (**A-H**) or low (**I-P**) GI diets were incubated with control serum (**E-H**, **M-P**) or α-MG-H1 (**A-D**, **I-L**). Deposition of MG-H1-modified protein (as indicated by a blue stain) was evaluated in unbleached (**C, D, G, H, K, L, O, P**) and bleached (**A, B, E, F, I, J, M, N**) sections, showing increased deposition in the outer nuclear (yellow

arrows), inner nuclear (white arrows), inner plexiform (orange arrows), ganglion cell (black arrows) and RPE (pink arrows) layers of the retinas from high GI-fed mice, corroborating prior Western blot analysis. Images in panels **B, D, F, H, J, L, N, P** were taken at a higher magnification to highlight changes in MG-H1 deposition in the RPE. GCL = ganglion cell layer; INL = inner nuclear layer; IPL = inner plexiform layer; ONL = outer nuclear layer; OS = outer segments; RPE = retinal pigment epithelium.

Figure 4. Localized damage to photoreceptor outer segments in 17- and 23.5-month-old mice fed a high GI diet. Electron micrographs of individual photoreceptor outer segments in mice from the 17-month-old high GI group (**A**), 17-month-old low GI group (**B**), 23.5-month-old high GI group (**C**), and 23.5-month-old low GI group (**D**). Yellow arrows indicate disorganization/vacuolization of outer segments. POS = photoreceptor outer segments; RPE = retinal pigment epithelium. Bar represents 2 μm.

Figure 5. Retinal nuclei in 17- and 23.5-month-old mice fed a high or low GI diet. Hematoxylin and eosin stained sections from 17-month-old high GI-fed (**A**), 23.5-month-old high GI-fed (**B**), 17-month-old low GI-fed (**C**), and 23.5-month-old low GI-fed (**D**) mice are shown. INL = inner nuclear layer; IPL = inner plexiform layer; IS = inner segments; ONL = outer nuclear layer; OPL = outer plexiform layer; OS = outer segments.

Figure 6. Comparable levels of AMD-like lesions observed in 17-month-old high GI-fed C57BL/6 mice in the absence or presence of HQ. Electron micrographs of retinas from mice fed high GI diets in the absence (**A**) or presence (**B**) of HQ. BLD = basal laminar deposit; BrM = Bruch's membrane (knobbed lines indicate thickness of Bruch's membrane); LIPO = lipofuscin-like granule; OCL = outer collagenous layer deposit; RPE = retinal pigment epithelium; V = vacuole; + indicates a loss of basal infoldings. Bar = 1 μm. Mean values for high GI-fed mice in the absence (blue bars) and presence (green bars) of HQ are shown for frequency of basal laminar deposits (**C**) and severity of basal laminar deposits (**D**). Error bars represent SEM. ** indicates $p < 0.05$ and # indicates $p < 0.1$.

3.8 Tables

Table 1. Diet Composition

Ingredient	High GI Diet (g/kg)	Low GI Diet (g/kg)
100% amylopectin (Amioca starch)	534	0
70% amylose/30% amylopectin (Hylon VII starch)	0	534
Casein	200	200
Sucrose	85	85
Soybean Oil	56	56
Wheat Bran	50	50
DL Methionine	2	2
Vitamin Mix	10	10
Mineral Mix	35	35
Hydroquinone	8	8
Macronutrient % of Total Energy		
Carbohydrate	65	65
Protein	21	21
Fat	14	14

Table 2. Frequency and Severity Grading Scheme for AMD-Like Lesions

Grading of Characteristics Evaluated by Frequency and/or Severity		
Characteristic	**Frequency Score (also see text)**	**Severity Score**
basal laminar deposits [90, 92, 118, 142]	number of deposits (including severity grades 1-3) per μm	0: no basal laminar deposits
		1: <1 μm with amorphous material
		2: <1 μm with fibrillar material OR >1 μm with amorphous material
		3: >1 μm with fibrillar material
basal laminar deposit-associated membranous debris [110, 143, 144]	number of debris per μm	N/A
cytoplasmic vacuoles [89, 90, 118]	number of vacuoles (including severity grades 1-3) per μm	0: no vacuoles
		1: empty vacuoles
		2: vacuoles contain granular debris
		3: vacuoles contain membranous debris or undigested photoreceptor outer segments
loss of basal infoldings [89, 145]	number of occurrences of absent basal infoldings (including severity grades 1-3) per μm	0: no absence of infoldings within 1 linear μm
		1: absence of infoldings within 1 linear μm
		2: periodic absence of infoldings, each within 1 linear μm OR an absence of infoldings of1-3 linear μm
		3: absence of infoldings of >3 linear μm
thickened Bruch's membrane [90, 92]	0: In each image, the average of the thickest and thinnest points of Bruch's membrane is < 0.4 μm. 1: In each image, the average of the thickest and thinnest points of Bruch's membrane is > 0.4 μm.	0: < 0.4 μm
		1: 0.4-1 μm and organized
		2: 0.4-1 μm and disorganized (loss of structure, vacuolization) OR > 1 μm and organized
		3: > 1 μm and disorganized
melanin [142, 146, 147]	number of melanosomes per μm	N/A
outer collagenous layer deposits [89]	number of deposits (including severity grades 1-3) per μm	0: no deposits
		1: < 0.5 μm at thickest point of deposit
		2: 0.5 - 1 μm at thickest point of deposit
		3: > 1 μm at thickest point of deposit
lipofuscin [118, 142, 148-150]	number of lipofuscin-like granules per μm	N/A

Table 3. Outer Nuclear Layer Thickness in 17- and 23.5-Month-Old Mice Fed a High or Low GI Diet

Rows of Outer Nuclei ± SEM		
Diet	Age	
	17 months	23.5 months
High GI	10.4 ± 0.8	8.5 ± 0.4†
Low GI	13.1 ± 1.3	8.5 ± 0.3*

* indicates a difference in mean thickness of outer nuclear layers between 17- and 23.5-month-old mice fed low GI diets with $p < 0.01$.

† indicates a difference in mean thickness of outer nuclear layers between 17- and 23.5-month-old mice fed high GI diets with $p = 0.06$.

Table 4. Inner Nuclear Layer Thickness in 17- and 23.5-Month-Old Mice Fed a High or Low GI Diet

Rows of Inner Nuclei ± SEM		
Diet	Age	
	17 months	23.5 months
High GI	5.2 ± 0.4	3.6 ± 0.0*
Low GI	5.9 ± 0.2	4.0 ± 0.2*†

* indicates a difference in mean thickness of inner nuclear layers between 17- and 23.5-month-old mice fed diets of the same GI with $p < 0.01$.

† indicates a difference in mean thickness of inner nuclear layers between 23.5-month-old mice fed diets of different GI with $p = 0.07$.

3.9 Figures

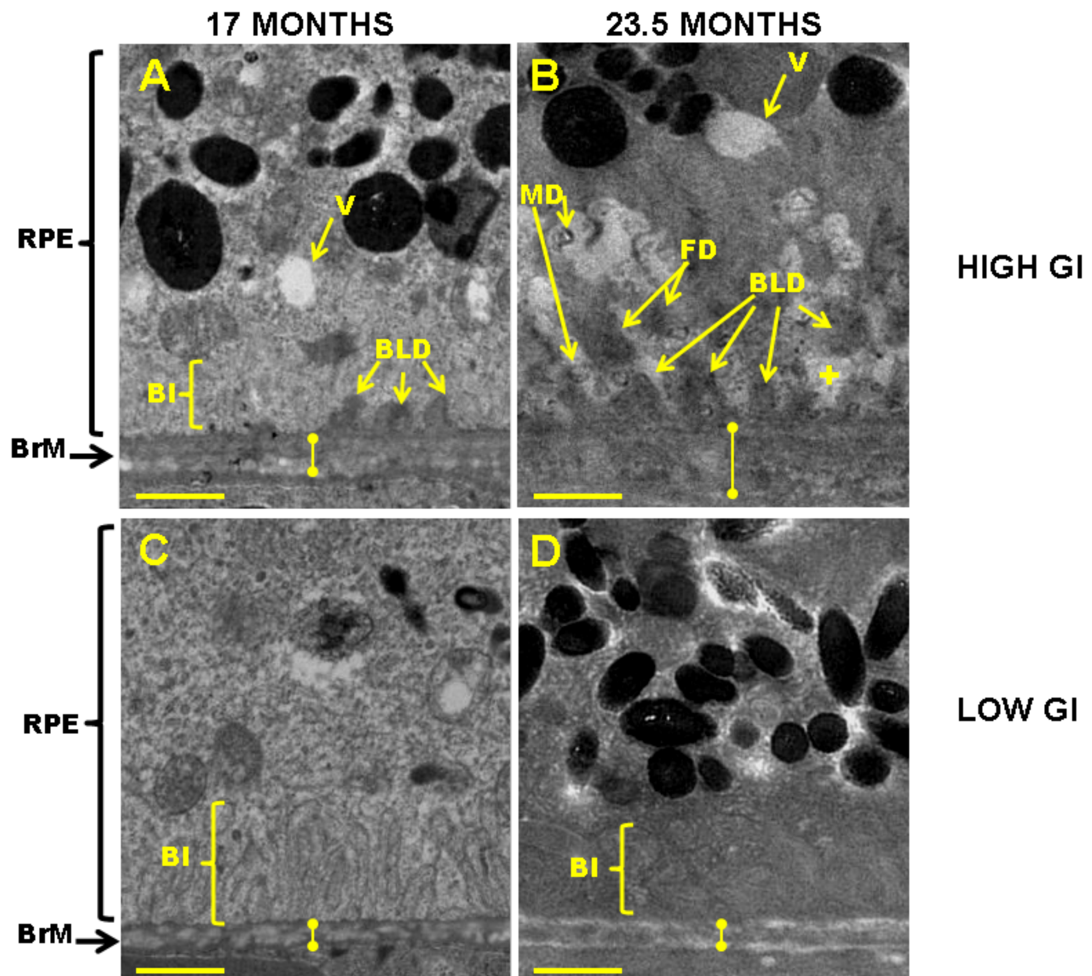
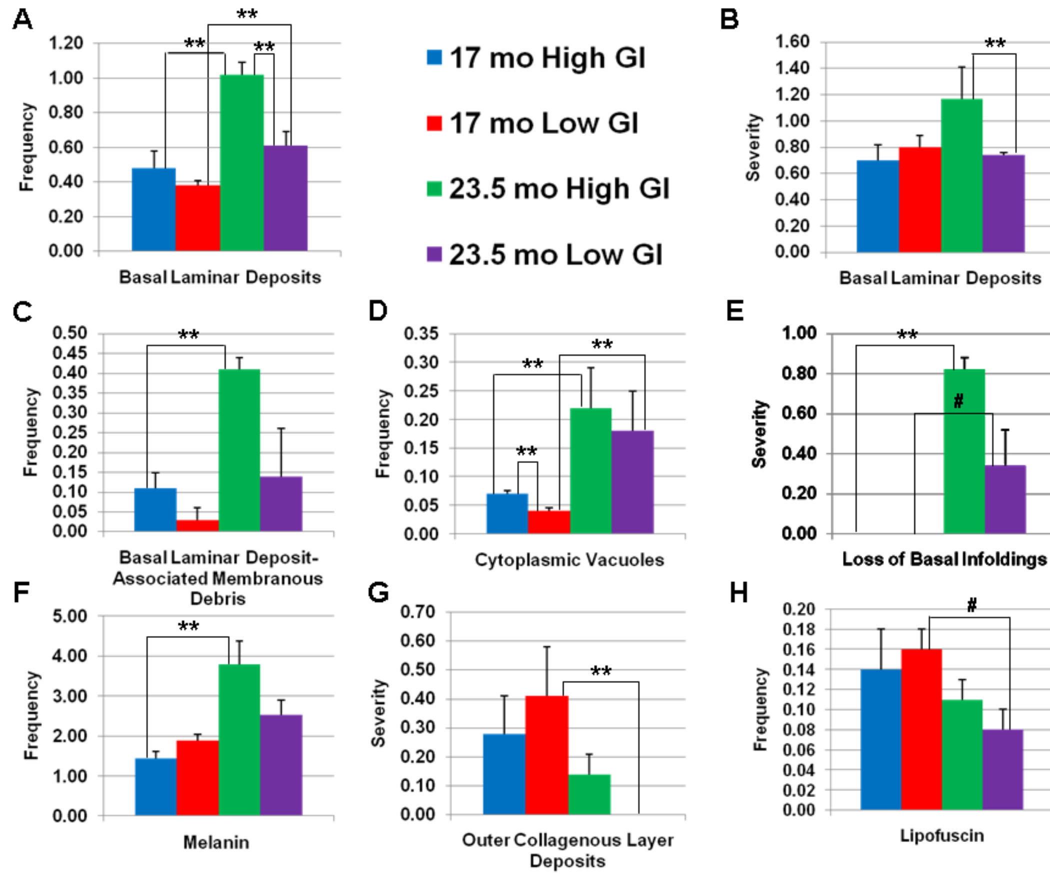


Figure 1.



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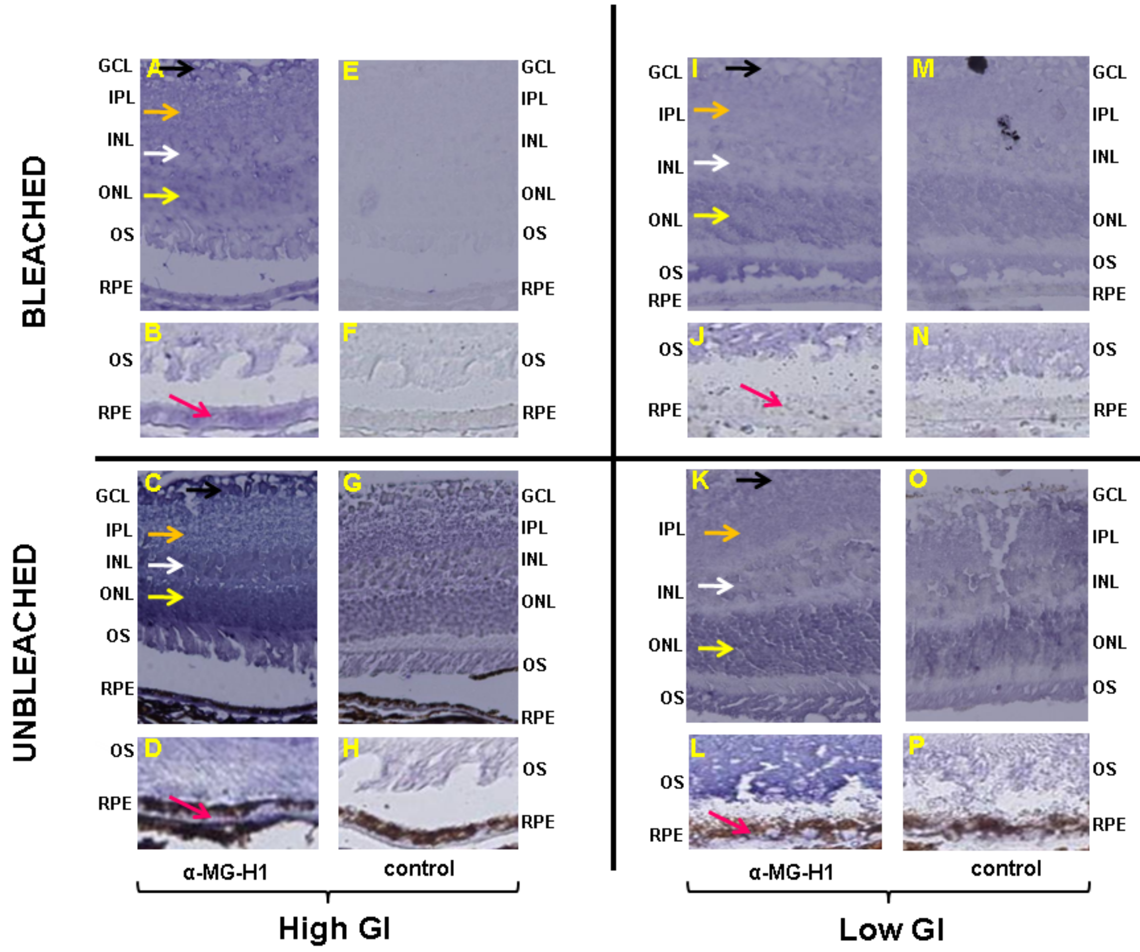

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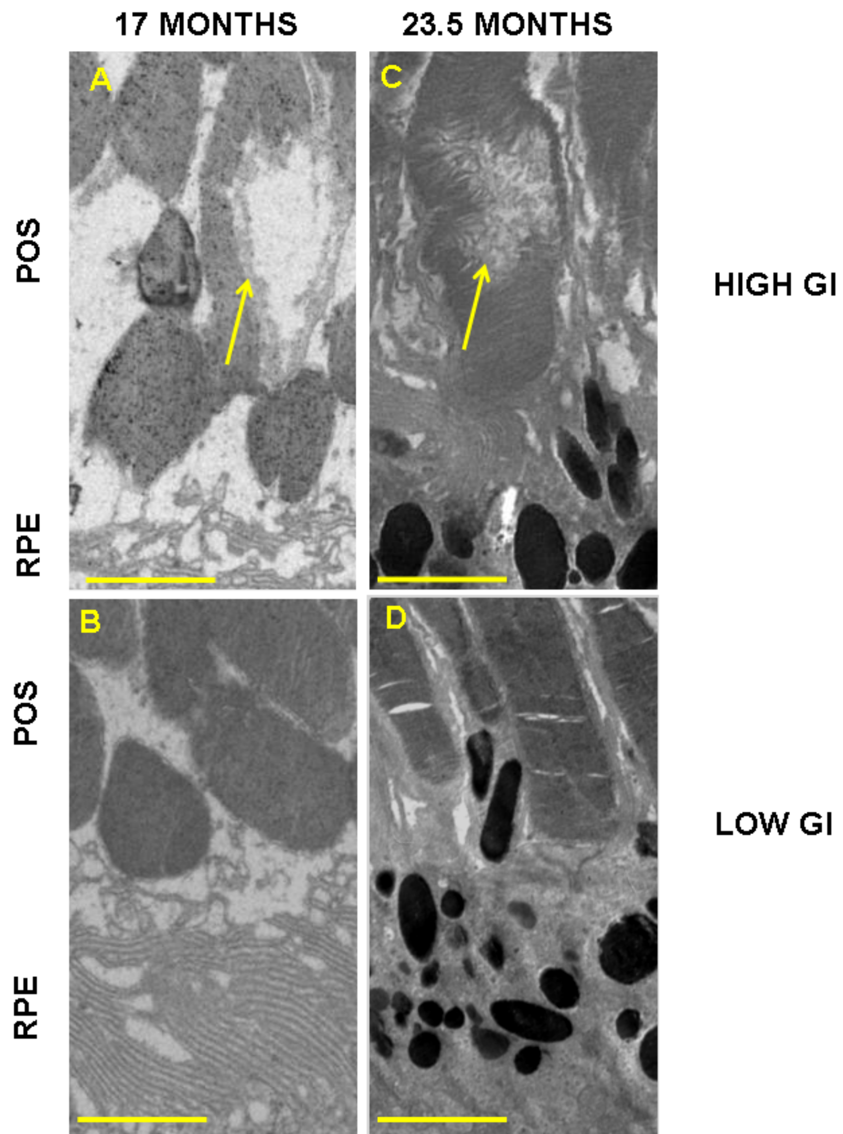

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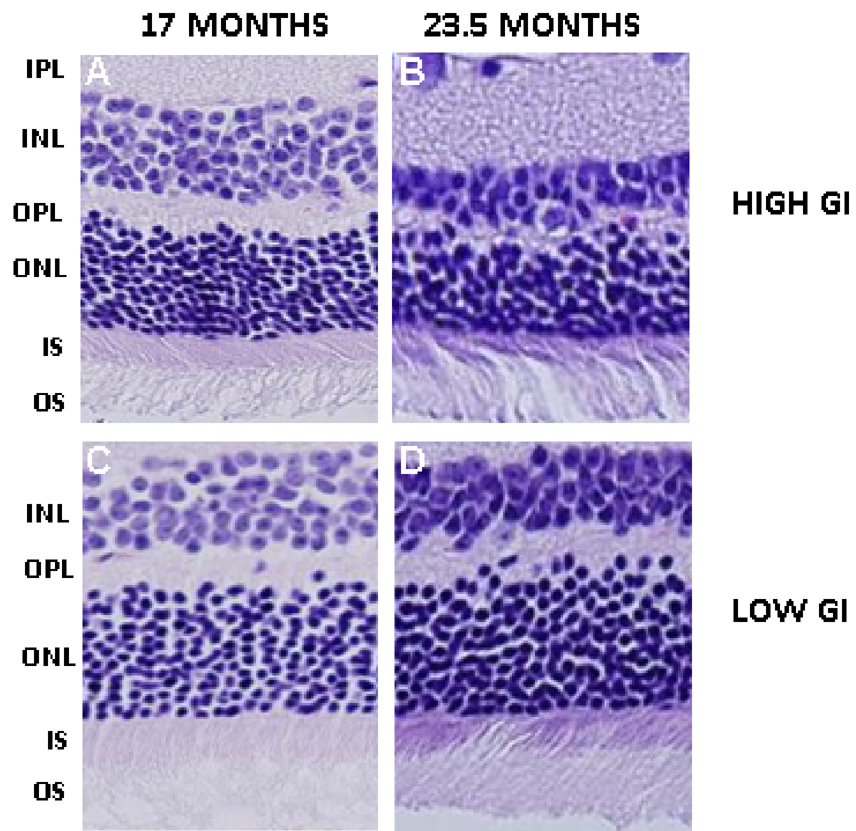

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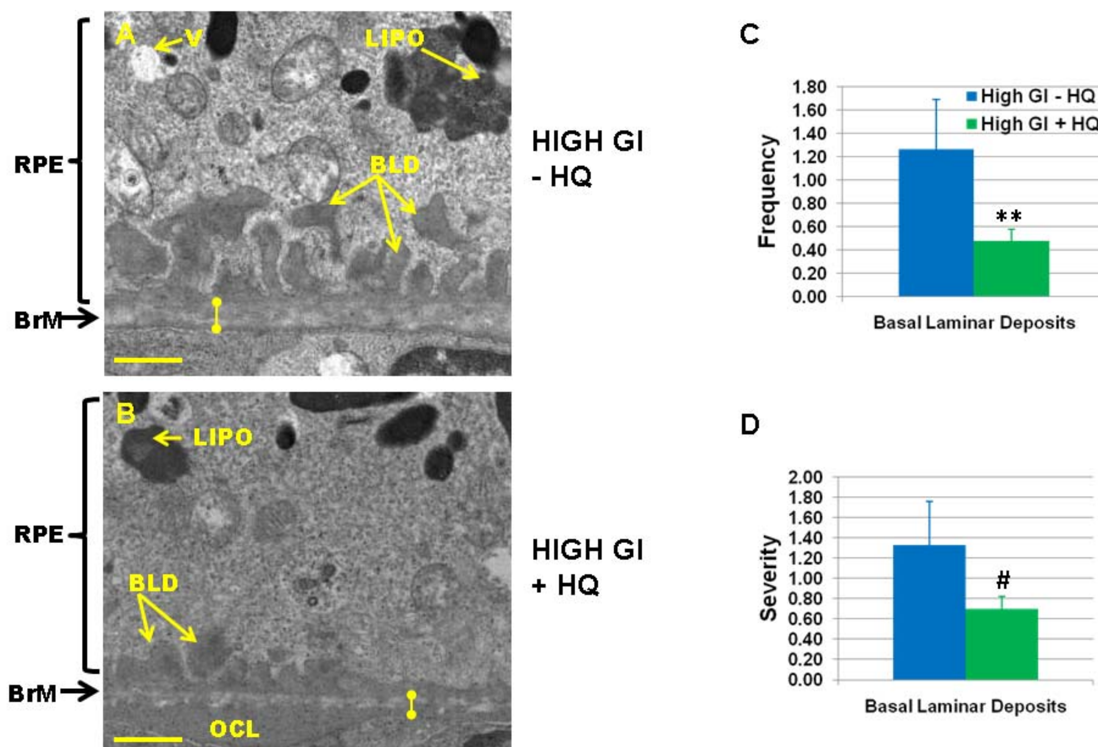


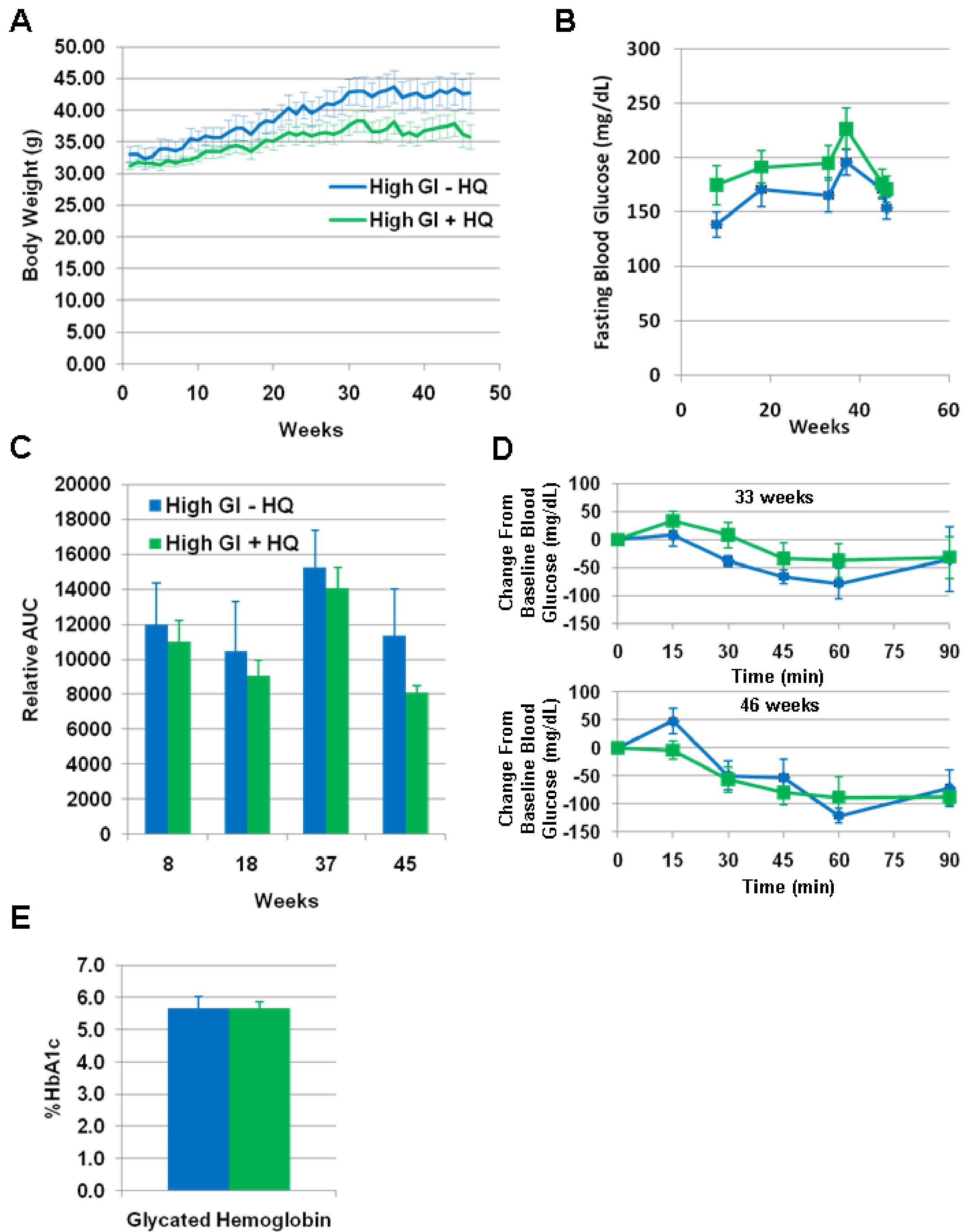
Figure 6.

3.10 Supplementary Material

Figure Legend and Figure

Supplementary Figure S1. Metabolic profile of 17 month-old C57BL/6 mice fed a high GI diet in the absence or presence of HQ. A) Body weights of mice fed a high GI diet without (blue line) and with (green line) HQ. **B**) Fasting blood glucose was measured in the groups fed a high GI diet without (blue line) and with HQ (green line). **C**) Area under the curve (AUC) for blood glucose during intraperitoneal oral glucose tolerance tests administered to mice fed high GI diets without (blue bars) and with HQ (green bars). AUC was calculated using the trapezoidal method. **D**) Changes from baseline blood glucose levels following injection of insulin during intraperitoneal insulin tolerance tests after 33 (top panel) or 46 (bottom panel) weeks of feeding. Blue line represents the non HQ-

exposed mice and green line represents the HQ-exposed mice. **E**) Levels of glycated hemoglobin in 17-month-old mice fed high GI diets without (blue bar) and with HQ (green bar). For all graphs, error bars represent SEM. Differences in body weight between groups were compared using Student's t test. Longitudinal changes in fasting glucose levels and glucose AUC during glucose and insulin tolerance tests were analyzed using two-way repeated measures ANOVA. Differences between groups at individual time points were compared post hoc using Student's t test. Differences in levels of glycated hemoglobin between diet groups were compared using Wilcoxon's Mann Whitney U test. For each parameter, there was no statistical difference between treatment groups.



Supplementary Figure S1.

Supplemental Methods

Fasting glucose and glucose tolerance test: Mice were fasted for 6 hours before an intraperitoneal glucose tolerance test. A clean razor blade was used to make

a horizontal cut in the lateral tail vein, releasing about 5 μl of blood that was applied to a OneTouch Ultra test strip in a OneTouch Ultra2 glucometer (Lifescan Inc, Milpitas, CA). This provided the fasting glucose level. After the fasting glucose was measured in each mouse, each mouse was injected intraperitoneally with 1 g/kg body weight D-(+)-glucose (≥99.5%, Sigma, St. Louis, MO) via a 25 5/8 gauge syringe. At 30, 45, 60, and 120 minutes after injection, the tail vein cut in each mouse was moistened to remove the clot and dried before release of another 5 μl of blood. This blood was applied to the test strip in the glucometer to measure the blood glucose level.

Insulin tolerance test: Mice were fasted for 6 h prior to intraperitoneal insulin tolerance test. A clean razor blade was used to make a horizontal cut in the lateral tail vein, releasing about 5 μl of blood that was applied to a OneTouch Ultra test strip in a OneTouch Ultra2 glucometer. This provided the fasting glucose level. After the fasting glucose was measured in each mouse, each mouse was injected intraperitoneally with 0.75 U/kg body weight Novolin R human insulin (Novo Nordisk Pharmaceutical, Princeton, NJ) via a 25 5/8 gauge syringe. At 15, 30, 45, 60, and 90 minutes after injection, the tail vein cut in each mouse was moistened to remove the clot and dried to release another 5 μl of blood. This blood was applied to the test strip in the glucometer to measure the blood glucose level.

Glycated hemoglobin: Erythrocytes were isolated as described above and glycated hemoglobin level was determined using a GLYCO-Tek Affinity Column

Kit (Helena Laboratories, Beaumont, Texas) as per the manufacturer's instructions. A new column was used for each sample.

Chapter 4. A High Glycemic Index Diet Alters Glucose Metabolism and Increases Cellular Stress

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Word Count: 7,797
Figures: 6
Tables: 1
Supplementary Figures: 2
Running Title: High Glycemic Index Increases Cellular Stress

Funding: This research was funded by USDA 1950-510000-060-01A, Johnson and Johnson Focused Giving, and NIH 13250, NIH 21212.

4.1 Abstract

Increasing rates of chronic diseases can be attributed to the alarming increase in simple sugar consumption over the last few decades. Diets that are high in these sugars are often of high glycemic index (GI), yet pathobiochemical mechanisms that relate dietary GI to risk for these age-related diseases have not been investigated in older animals. Increased risk for these diseases has been linked to accumulation of advanced glycation end products (AGEs). To investigate the mechanism by which high GI diets may hasten age-related disease, we measured fasting glucose and insulin levels, glucose clearance, and accumulation of AGEs in kidneys, hearts and brains of 17- and 23.5-month-old C57BL/6 mice fed high or low GI diets. To determine if AGE accumulation was related to impairment of protein clearance, we measured the effect of glycative stress on 20S proteasome activity. We found that consumption of the higher GI diet impaired glucose tolerance and increased insulin resistance at both ages. At 23.5 months of age, AGEs accumulated in multiple tissues of higher GI-fed mice. Higher GI diets and higher levels of AGEs were associated with lower proteasome activity in heart but, unexpectedly, higher activity in kidney and brain. We demonstrated that a low GI diet protects against compromises in blood glucose and insulin levels. This diet also prevented accumulation of tissue AGEs in older mice. Older mice fed a high GI diet are an excellent model to test drugs for prevention of chronic diseases that are associated with accumulation of oxidized or damaged proteins.

4.2 Introduction

Consumption of high glycemic index (GI) diets is associated with increased risk for chronic diseases such as type II diabetes, renal dysfunction, cardiovascular disease and neurodegenerative disease (1-4). These chronic diseases affect nearly 50% of adults and are associated with approximately 70% of deaths in America (5-7). The dramatic increase of simple sugars in the American diet over the last several decades has raised alarm. This results in increased dietary GI, creating an urgent need to model and understand relationships between dietary GI, metabolism and pathobiology (8, 9). The glycemic index (GI) of a particular food quantifies the rise in blood glucose following the consumption of 50 grams of carbohydrate from that same food, relative to the rise in blood glucose following the consumption of 50 grams of carbohydrate from a standard food (glucose, white bread). Foods with a high GI contain carbohydrates that are more readily broken down than carbohydrates found in foods with a low GI. Thus, high GI foods induce a larger increase in blood glucose levels than low GI foods (10).

Despite the observational data linking GI with age-related disease, pathobiochemical mechanistic explanations for the harmful effects of a high GI diet, and conversely, the benefits of a low GI diet, are lacking. Laboratory rodents are essential tools to explore these mechanisms, but previous studies investigating associations between dietary GI and these age-related diseases were done using only young rodents (11-16). Physiological and biochemical changes observed in older animals would more closely mimic human aging and

provide critical insights into etiological relationships between GI modulation, physiology, cell biology, biochemistry and aging in elderly humans.

AGEs are proteins that are non-enzymatically modified by glycating agents such as glucose or its metabolites. Often, these sugar derivatives are modified by glucose on lysine and arginine residues of proteins (17). Associations between consumption of diets of different GIs and AGE levels in blood have been explored, but literature regarding effects of dietary GI on AGE accumulation in tissues is very limited (18, 19). Accumulation of AGEs in tissues is observed in many chronic diseases and has been associated with impaired organ function (17, 20-23). In the kidney, an organ involved in clearance of blood-borne AGEs, AGE accumulation is associated with renal failure (24, 25). AGE cross-links in the vessel wall also induce vascular stiffness and are associated with atherosclerosis and decreased heart function (26-31). In neurons, AGE accumulation is toxic and is associated with dementia (32-34). These results suggest etiologic roles for AGEs in disease.

Levels of AGEs are determined by rates of formation and proteolytic removal. The ubiquitin proteasome system (UPS) is one of the cellular mechanisms responsible for protein degradation and is also known to be modulated by oxidative stress (35, 36). Ubiquitin is a small, 8 kDa protein that attaches to other proteins to form multi-ubiquitin chains. These chains, known as ubiquitin conjugates, can target substrates for degradation by the proteasome (37). In cells exposed to glycative stress, toxic AGEs accumulate and UPS-mediated degradation of substrates is impaired. However, proteasome activity

has not been shown to be altered (Uchiki et al., 2011, *in press*) (38). Changes in proteasome activity due to a glycative stress such as a high GI diet have not been measured in animal tissues.

Having observed that GI modulates risk for diseases that afflict the elderly, we hypothesized that consumption of a low GI diet would confer the greatest benefit to older animals. To test this hypothesis, we characterized glucose and insulin metabolism and the accumulation of AGEs in the kidneys, hearts and brains of 17- and 23.5-month-old mice fed high or low GI diets. We also measured proteasome activity in these tissues from 23.5-month-old mice. Each of these tissues is either involved in the metabolism of AGEs, or is a site of AGE accumulation during disease (17, 20). These data provide insight regarding the most useful models in which to study the association between dietary GI and pathobiology of disease.

4.3 Methods

Ethics statement

This study was carried out and approved under the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University IACUC protocols and in accordance with the Animal Welfare Act provisions and all other animal welfare guidelines such as the NIH Guide for the Care and Use of Laboratory Animals.

Animals

Five and 16-month-old male C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, MA). The 5-month-old mice were fed either high (n=10) or low (n=10) GI diets for 46 weeks (**Table 1**). They were killed at 17 months of age and are hereafter referred to as the 17-month-old group. We also fed 16-month-old mice high (n=10) or low (n=10) GI diets for 26 weeks. They were killed at 23.5 months of age and are hereafter referred to as the 23.5-month-old group. Mice were weaned onto their high or low GI diets over a 6-week period, becoming fully acclimated to their diets by 6.5 and 17.5 months of age, respectively (**Figure 1**).

In both age groups, the mice were pair-fed to ensure equal consumption between diet groups. The high and low GI diets were isocaloric and of identical macronutrient distribution. The only difference between the high and low GI diets was the distribution of starch (100% amylopectin in the high GI diet, and 30% amylopectin/70% amylose in the low GI diet). All of the diets used in this study were formulated by Bio-Serv (Frenchtown, NJ). National Starch (Bridgewater, NJ) generously donated Amioca starch (100% amylopectin) for incorporation into the high GI diet, and Hylon VII starch (30% amylopectin/70% amylose) for incorporation into the low GI diet.

In the 17-month-old mice, glucose was measured at 8.25, 10.5, 14, 15, 16.75 and 17 months of age, after 8, 18, 33, 37, 45 and 46 weeks of consuming the experimental diets, respectively. Week 0 refers to the first week after the 6-week diet acclimation period. Glucose tolerance tests were performed at 8.25, 10.5, 15 and 16.75 months of age. Insulin tolerance tests were performed at 14

and 17 months of age, after 33 and 46 weeks of diet consumption, respectively. Just prior to being killed, blood was collected via cardiac puncture to measure insulin and glycated hemoglobin levels. In the 23.5-month-old mice, blood glucose was measured at 19.5, 21.75 and 23.5 months of age, after 8, 18 and 26 weeks of consuming the experimental diets, respectively. Glucose tolerance was measured at 19.5 and 21.75 months of age, and blood was collected via cardiac puncture just prior to being killed for analysis of insulin levels.

All mice were feed-deprived 6 hours prior to being euthanized with carbon dioxide, and killed by cervical dislocation. Tissues were then collected and frozen in liquid nitrogen for storage at -80°C.

Blood Measurements

Glucose levels and glucose tolerance test: Mice were feed-deprived for 6 hours before an intraperitoneal glucose tolerance test. A clean razor blade was used to make a horizontal cut in the lateral tail vein, releasing about 5 μl of blood that was applied to a OneTouch Ultra test strip in a OneTouch Ultra2 glucometer (Lifescan Inc, Milpitas, CA). This provided the feed-deprived glucose level. After the feed-deprived glucose level was measured in each mouse, each mouse was injected intraperitoneally with 1 g/kg body weight D-(+)-glucose (≥99.5%, Sigma, St. Louis, MO) via a 25 5/8 gauge syringe. At 30, 60 and 120 minutes after injection, the tail vein cut in each mouse was moistened to remove the clot and dried before release of another 5 μl of blood. This blood was applied to the test

strip in the glucometer to measure the blood glucose level. Area under the curve (AUC) was calculated using the trapezoidal method.

Insulin tolerance test: Mice were feed-deprived for 6 hours prior to the intraperitoneal insulin tolerance test. A clean razor blade was used to make a horizontal cut in the lateral tail vein, releasing about 5 μl of blood that was applied to a OneTouch Ultra test strip in a OneTouch Ultra2 glucometer. This provided the feed-deprived glucose level. After the feed-deprived glucose level was measured in each mouse, each mouse was injected intraperitoneally with 0.75 U/kg body weight Novolin R human insulin (Novo Nordisk Pharmaceutical, Princeton, NJ) via a 25 5/8 gauge syringe. At 15, 30, 45, 60 and 90 minutes after injection, the tail vein cut in each mouse was moistened to remove the clot and dried to release another 5 μl of blood. This blood was applied to the test strip in the glucometer to measure the blood glucose level.

Feed-deprived insulin levels: Blood was collected via cardiac puncture and stored on ice in K_2EDTA-coated Vacutainer tubes (BD Biosciences). Whole blood was spun at 4°C at 4000 RCF for 10 minutes. Supernatant was removed and analyzed for insulin levels using the UltraSensitive Mouse Insulin ELISA kit (Crystal Chem, Inc., Chicago, IL) as per the manufacturer’s instructions. Remaining plasma samples and erythrocytes were stored at -80°C.

HOMA-IR: HOMA-IR values were calculated from feed-deprived glucose and insulin levels in the mice just prior to being killed. HOMA-IR = (mmol/L glucose x μU/ml insulin) / 22.5.

Glycated hemoglobin: Erythrocytes were isolated as described above and glycated hemoglobin level was determined using a GLYCO-Tek Affinity Column Kit (Helena Laboratories, Beaumont, Texas) as per the manufacturer's instructions. A new column was used for each sample.

Western Blot Analyses

Once the mice were killed, tissues were snap frozen and stored at -80°C. For analysis, tissues were ground and homogenized in buffer containing 1% NP-40, 10 mM Tris-Cl pH 7.5, 100 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml AEBSF and 10 mM N-ethylmaleimide. For assessment of relative levels of MG-H1-modified protein in kidneys, hearts and brains, the homogenized tissues were resolved on SDS-PAGE, transferred to nitrocellulose and stained with Ponceau S (Sigma, catalog #P7170) to evaluate loading and transfer efficiency. Membranes were then incubated with anti-MG-H1 antibody that was generously provided by M. Brownlee (Albert Einstein College of Medicine, NY). After incubation with HRP-conjugated anti-mouse secondary antibody, the antigen was visualized using a Super Signal chemiluminescence detection kit (Thermo Scientific, Waltham, MA).

For analysis of carbonyls, samples were derivitized with dinitrophenylhydrazine (DNPH) by incubating each sample with 5 mM DNPH (total volume 20 µl) for 15 minutes at room temperature out of direct light exposure. Forty µl 2 M Tris was added to stop the reaction, and samples were then resolved on SDS-PAGE, transferred to nitrocellulose, stained with Ponceau S and incubated with anti-DNP antibody (Sigma, catalog # D9656) to detect protein-bound hydrazones (39). After incubation with HRP-conjugated anti-rabbit antibody, the antigen was visualized using a Super Signal chemiluminescence detection kit (Thermo). Densitometric analysis of western blots was carried out using Image J (NIH, Bethesda, MD).

Proteasome Peptidase Activity Assay

One hundred µg of tissue lysate (homogenized as stated above) was incubated in a buffer of 50 mM tris-HCl pH 8.0, 100 mM NaCl, 5 mM EDTA, 1 mM EGTA, 3 mM NaN_3, 0.04% 3-[(3-chloramidopropyl)dimethylammonio]-1-propane sulfonate, and 0.1 M DTT, along with Succinyl-Leu-Leu-Val-Tyr-amidomethylcoumarin (LLVY-AMC) (fluorogenic peptide to measure chymotrypsin-like activity) in the presence or absence of 97 µM MG132 (40). The final concentration of peptide substrate was 48.5 µM and the final volume of the reaction mixture was 200 µl. Enzymatic kinetics were measured with a temperature-controlled microplate fluorometric reader (25°C) over five 3-minute cycles. Excitation/emission wavelengths were 380/440 nm. After subtracting fluorescence readings of samples incubated with MG132 from readings of

samples without MG132, activity was determined by measuring the relative change in fluorescence between cycles.

Statistics

Differences in body weight at sacrifice were compared between groups using Student's t test. Longitudinal changes in fasting glucose levels and glucose AUC during glucose and insulin tolerance tests were analyzed using two-way repeated measures ANOVA. Differences between diet groups at individual time points were compared post hoc using Student's t test. Differences in fasting insulin and HOMA-IR levels were compared between diet groups using Student's t test after the data were log transformed, and differences in levels of glycated hemoglobin between diet groups were compared using Wilcoxon's Mann Whitney U test. Differences in mean accumulation of MG-H1-modified protein and protein carbonyls, as well as levels of proteasome activity were compared between diet groups using Wilcoxon's Mann-Whitney U test. Bonferroni's adjustment was used to account for multiple comparisons. All statistical comparisons were made using (SAS 9.2, Cary, NC).

4.4 Results

4.4.1 Consumption of a Higher GI Diet Increases Metabolic Stress in Middle-Aged and Older Mice

High GI diets have been associated with metabolic stress, raising glucose and insulin levels in younger animals (11-13, 15, 16). To expand upon these

findings, we measured body weight, glucose levels, and insulin levels in high and low GI-fed mice up to 17 months of age (**Figure 2**). At baseline (5-months-old) and after becoming fully acclimated to the experimental diets (6.5-months-old), there was no statistical difference in body weight between diet groups. While the low GI-fed mice maintained a relatively constant weight, the weight of mice that consumed the high GI diet for 46 weeks increased by 14% over the course of the study (**Figure 2A**). Thus, at sacrifice at 17 months of age, high GI-fed mice were 24.7% heavier than low GI-fed mice ($p < 0.05$). This observation is surprising since both diets are > 90% digestible, and the mice were pair-fed to achieve equivalent food consumption between groups (16). Similar weight gains in high GI-fed mice have been reported, but this is typically accompanied by weight gains in low GI-fed mice as well (14, 16).

Consistent with data from younger animals, feed-deprived blood glucose levels were higher in the 17-month-old high GI-fed mice compared to age-matched low GI-fed mice ($p < 0.05$ at 8.25 and 17 months of age, after 8 and 46 weeks on experimental diets, respectively) throughout the duration of the study (**Figure 2B**) (13). Glucose levels in the high GI-fed mice were slightly higher than those reported in chow-fed mice of similar age, but this increase may be attributable to our use of a 6hr period without food, rather than an overnight food deprivation period (41). Glucose tolerance tests indicated that the 17-month-old high GI group had impaired glucose tolerance compared to the age-matched low GI group over the duration of the study ($p < 0.05$ at 8.25, 10.5, 15 and 16.75 months of age, after 8, 33, 37 and 45 weeks on diet, respectively) (**Figure 2C**).

Although we observed higher AUC levels in both diet groups than those reported by Isken et al., our observation of a GI-related difference in blood AUC corroborates their results from 4- and 11- month-old mice fed similar diets (16). Elevated fasting blood glucose and impairment of glucose tolerance could be due to a decreased sensitivity of peripheral cells to insulin (42). To determine if the high GI-fed 17-month-old mice were peripherally insulin resistant, insulin tolerance tests were administered at 14 and 17 months of age, after 33 and 46 weeks of consuming study diets. The data, documented as change from baseline blood glucose, show that like younger mice, both diet groups responded similarly to insulin (**Figure 2D**) (16). At 17 months of age, feed-deprived insulin levels in the high GI-fed mice were similar to those reported for chow-fed mice and higher than those observed in our low GI-fed mice ($p < 0.05$) (**Figure 2E**) (41). HOMA-IR values were calculated from the feed-deprived glucose and insulin levels and show that relative to the low GI-fed mice, the 17-month-old high GI-fed mice were more insulin resistant ($p < 0.05$) (**Figure 2F**).

Glycated hemoglobin is an indicator of long-term exposure to glucose or its metabolites and in humans has been associated with insulin resistance (43, 44). Glycated hemoglobin is also susceptible to further oxidative modification, resulting in AGE formation (45). 17-month-old mice fed a high GI diet had levels of glycated hemoglobin that were nearly twice as high as those in age-matched mice fed a low GI diet ($p < 0.05$) (**Figure 2G**). Since the higher GI-fed mice were also more insulin resistant, our finding is consistent with the association between insulin resistance and protein glycation in humans.

A second group of mice was maintained until 23.5 months of age in order to repeat and extend these measures to elderly mice. These mice consumed standard laboratory chow for their first 16 months. Both diet groups had comparable body weights when they began the high or low GI diet. Unexpectedly, the high GI group lost 7.5% of their body weight over the course of the 26 weeks that they consumed their diet. The low GI group lost approximately 21% of their weight during this time (**Figure 3A**). The majority of the weight loss in the low GI group occurred during the first 10 weeks of consuming the low GI diet. Similar to the weight difference observed in the 17-month-old animals, the 23.5-month-old low GI group weighed about 22% less than the age-matched high GI group ($p < 0.05$). The significantly lower body weights of the 17- and 23.5-month-old lower GI-fed mice as well as the weight loss observed in the 23.5-month-old low GI-fed mice contrast with results from other mouse studies using similar diets (11-13, 15, 16).

Among 23.5-month-old mice, feed-deprived blood glucose levels at 19.5 and 21.75 months of age (after 8 and 18 weeks of consuming the experimental diets) were significantly higher in the high GI group ($p < 0.05$) (**Figure 3B**). Although the blood glucose levels in the high-GI group remained higher than those in the low GI group, this difference was not significant at 23.5 months of age. Feed-deprived blood glucose levels in the high GI-fed 23.5-month-old mice were similar to those reported for similarly aged chow-fed mice, but were not as high as those observed in the high GI-fed 17-month-old-mice at all time points (**Figure 2**) (46-48). Glucose tolerance tests in the older mice showed a greater

area under the curve (AUC) of blood glucose in the high GI group compared to the low GI group at 19.5 months of age (after 8 weeks of diet consumption), and this difference remained at 23.5 months of age ($p < 0.06$) (**Figure 3C**). The feed-deprived insulin levels observed in the high GI-fed mice were typical for mice 23.5 months of age, but the levels in the low GI group were remarkably low (46-48). At 23.5 months of age, insulin levels in the low GI group were less than half of those in the high GI group ($p < 0.05$) (**Figure 3D**). The relative insulin resistance experienced by the high GI-fed mice, as reflected by their HOMA-IR values, are nearly 3 times that of the low GI-fed mice ($p < 0.05$) (**Figure 3E**). This suggests that the relative difference in insulin resistance between diet groups is driven by the remarkably low glucose and insulin levels in the low GI-fed mice.

In both age groups, the relative insulin sensitivity and improved glucose tolerance of the low GI-fed mice may also involve differences in diet-associated changes in body weights. Low GI-fed mice had lower body weights than the high GI-fed mice. Lower body weights are often associated with lower glucose and insulin levels and improved glucose tolerance.

4.4.2 Effect of Dietary GI on Accumulation of AGEs and Protein Carbonyls in Tissue

Having observed relatively more severe glucose intolerance and insulin resistance in higher GI-fed mice of both age groups, we suspected that the higher GI diet would also increase glycative stress within tissues. Thus, we

measured levels of AGEs in the kidneys, hearts and brains of these mice. We used accumulation of MG-H1-modified protein as our primary indicator of AGEs because it is formed upon the reaction of proteins with methylglyoxal, one of the most biologically prevalent and reactive sugar metabolites (49). MG-H1-modified proteins have also been observed to increase in tissues with age (Uchiki et al, 2011, *in press*). In our analysis, we focused on MG-H1-modified protein species ≥ 60 kDa because these moieties are often integrated into aggregates that are considered cytotoxic (50). Also, detection of higher molecular weight species is less likely to be confounded by non-specific detection of endogenous mouse IgG. Consistent with our prior observation that aging is associated with exacerbated accumulation of AGEs (Uchiki et al, 2011 *in press*), robust GI-related differences in levels of AGEs were observed in 23.5-month-old mice. Western blots revealed that in the kidneys, there was greater accumulation of AGEs in high GI-fed compared to low GI-fed mice ($p = 0.05$) (**Figure 4A, B**). A similar trend was observed in the hearts (**Figure 4C, D**) and brains (**Figure 4E, F**). The 17-month-old high GI-fed mice also tended to have more AGE accumulation in the kidneys, hearts and brains than in low GI-fed mice albeit differences between diet groups in these middle-aged mice were not statistically significant due to large inter-individual variability within the diet groups (**Supplementary Figure S1**).

Using antibodies that detect protein carbonyls, we tested whether the higher GI diet exerted a more generalized oxidative insult in tissues of 17-month-old mice (51). Impressively, consumption of the higher GI diet was associated with approximately 3-fold higher levels of protein carbonyls in the kidneys and

brains ($p < 0.05$) (**Figure 5**) of these mice. Collectively, these data suggest that there is increased systemic oxidative stress, as indicated by elevated levels of protein carbonyls and/or AGEs in multiple tissues in animals that consumed the higher GI diet.

4.4.3 Effect of Dietary GI on 20S Proteasome Activity

As noted earlier, AGEs may accumulate because rates of formation exceed rates of removal. Glycated proteins are not as readily recognized by the UPS. Furthermore, cellular glycative stress limits proteolytic capacity. To determine if high GI-induced oxidative stress impaired proteasome activity, we measured 20S proteasome activity in 23.5-month-old mice fed high or low GI diets. Consistent with our hypothesis of GI modulating UPS activity, 20S proteasome activity was lower in the hearts of mice fed the high GI diet compared to age-matched mice fed the low GI diet ($p < 0.05$) (**Figure 6A**). However, this observation was not generalizable across tissues. Surprisingly, proteasome activity was slightly higher in the kidneys and significantly higher in the brains ($p < 0.05$) of higher GI-fed mice (**Figure 6B, C**).

4.4.4 Association Between AGEs and 20S Proteasome Activity in Individual Mice

Although proteasome activity revealed significant inter-individual variability, the data above suggest that within an animal there is an inverse association between proteasome activity and accumulation of AGEs in certain tissues. To glean more information regarding the relationship between AGE

accumulation and the UPS, AGE accumulation and proteasome activities in individual mice were compared. Three animals were available for these assays. **Supplementary Figure S2** depicts AGE accumulation and the corresponding proteasome activity in the kidney and heart of individual 23.5-month-old mice. Mouse “H1” and “H2” were fed the high GI diet and “L1” was fed the low GI diet. In these tissues, we observed an inverse relationship between levels of AGEs and proteasome activity (**Supplementary Figure S2**). This suggests that AGEs may be substrates for degradation and/or that there is insufficient proteolytic capacity to remove the AGEs from these tissues. This inverse relationship was not observed in the brain.

4.5 Discussion

Uncertainty regarding the metabolic and cellular consequences of stresses imposed by a high GI diet on older animals prompted us to explore the effects of high and low GI diets on glucose and insulin levels in 17- and 23.5-month-old mice. We also monitored tissue levels of AGEs and proteasome activity because the relationship between GI and these indicators of cellular stress has not been explored. Consistent with data from studies that used younger mice, we found that consuming lower GI diets protected against age-related stresses that are thought to be causally related to chronic disease (13, 16). Importantly, these advantages of consuming lower GI diets were observed in later life. Together, the data confirm human epidemiologic data and validate the salutary effects of consuming lower GI diets (2, 3, 52, 53). The data also

help rationalize the need for intervention trials to test if lower GI diets slow progress of age-related disease.

The 17- and 23.5-month-old mice exhibited more severe glucose intolerance, as indicated by higher AUC values, than comparably fed younger mice (**Figures 2, 3**) (16). In addition, consuming the high GI diet as compared to the low GI diet was associated with higher AUC values in both 17- and 23.5-month-old mice (**Figures 2, 3**). More severe insulin resistance was also observed in the higher GI-fed mice of both age groups (**Figures 2, 3**). We did not observe a difference in insulin resistance between 17- and 23.5-month-old mice fed the same diet. Despite the increased AUC values and compromised insulin sensitivity in the high GI-fed groups, none of the mice in this study became diabetic. Glucose and insulin levels were lower than those reported in diabetic mice (54, 55). In fact, the glucose and insulin levels of the high GI-fed mice resembled those of healthy chow-fed mice, while consumption of the low GI diet protected mice against the expected age-related increases in feed-deprived glucose and insulin levels. This low GI-mediated protection was more obvious in the older mice. Indeed, the glucose and insulin levels in mice fed the lower GI diet were lower than levels that are often observed in similarly aged chow-fed mice (46, 47). The advantages imparted by the low GI diet appear to be due in part to the higher amount of resistant starch in their diets compared to standard chow and the high GI diet (56, 57). Increasing amounts of resistant starch have shown to improve insulin sensitivity (58, 59). Also, low glucose and insulin levels may be mediated by the lower body weights of lower GI-fed mice. The

differences in body weight between diet groups that we observed in the 17- and 23.5-month-old mice are unprecedented. The discrepancy between our results and those of previous GI interventions may be due to the advanced age of the mice in this study and this underscores the need to work with aged mice (11-14, 16).

Obesity is a risk factor for many chronic diseases including age-related macular degeneration, cardiovascular disease, metabolic syndrome and diabetes (60-62). The absence of weight gain in the 17-month-old low GI group and the weight loss in the 23.5-month-old low GI group, suggests that a low GI diet may be used as an effective weight-loss tool. Presumably, this diet would be beneficial for those suffering from chronic diseases in which obesity is a contributing risk factor.

Intracellular levels of AGEs a) are considered markers of chronic stress, b) have been reported to accumulate with increased age and c) this accumulation is toxic in various cells (20, 63-67). Our data indicate that consumption of a low GI diet confers protection against accumulation of AGEs, as observed most readily in the 23.5-month-old mice. The prolonged chronic oxidative insults associated with aging contribute to AGE accumulation in tissue, and this further augments cellular stress (17, 20). Accumulation of AGEs and protein carbonyls in multiple tissues of higher GI-fed mice indicates that the higher GI diet induces systemic oxidative, specifically glycative, stress in tissue, in addition to increasing glycation of blood proteins (**Figures 2G, 4, 5**).

It has been shown that upon mild stress, proteasome activity increases, while prolonged, chronic stress can cause proteasome activity to decrease (68). In retinal pigment epithelial cells, glycative stress did not alter proteasome activity, but the effects of consuming high or low GI diets on proteasome activity in mammalian tissues have not been documented (Uchiki et al, 2011 *in press*). We found that higher GI-fed mice had less proteasome activity in the heart (**Figure 6A**). This is consistent with data from individual mice that suggest that insufficient proteolytic capacity is associated with accumulation of AGEs (**Supplementary Figure S2**). However, proteasome activity was higher in the brain and perhaps the kidney of higher GI-fed mice (**Figure 6B, C**). The hypothesis to be tested in a future larger study is that consuming lower GI diets protects against proteasome inactiviation.

In summary, this GI intervention study has demonstrated that low GI diets confer protection from expected age-related increases in glucose and insulin levels and accumulation of AGEs in middle-aged and, most clearly, in older mice. Thus, older rodents appear to be a new model in which to study the benefits of consuming lower GI diets, and for testing of dietary or pharmaceutical interventions. The association between systemic accumulation of AGEs and protein carbonyls, and diminished proteasome activity suggests that maintenance of efficient UPS-mediated degradation of AGEs may be one way in which low GI diets reduce risk for chronic disease.

4.6 References

1. Murakami K, Miyake Y, Sasaki S, Tanaka K, Fukushima W, Kiyohara C, Tsuboi Y, Yamada T, Oeda T, et al. Dietary glycemic index is inversely associated with the risk of Parkinson's disease: a case-control study in Japan. Nutrition. 2010 May;26:515-21.

2. Jenkins DJ, Kendall CW, McKeown-Eyssen G, Josse RG, Silverberg J, Booth GL, Vidgen E, Josse AR, Nguyen TH, et al. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. Jama. 2008 Dec 17;300:2742-53.

3. McKeown NM, Meigs JB, Liu S, Rogers G, Yoshida M, Saltzman E, Jacques PF. Dietary carbohydrates and cardiovascular disease risk factors in the Framingham offspring cohort. J Am Coll Nutr. 2009 Apr;28:150-8.

4. Gopinath B, Harris DC, Flood VM, Burlutsky G, Brand-Miller J, Mitchell P. Carbohydrate nutrition is associated with the 5-year incidence of chronic kidney disease. J Nutr. 2011 Mar;141:433-9.

5. Kung H, Hoyert D, Xu J, Murphy S. Deaths: final data for 2005. National Vital Statistics Reports. 2008;56.

6. Wu S, Green A. Projection of chronic illness prevalence and cost inflation. Santa Monica, CA: RAND Health; 2000.

7. Aging Ao. Aging Statistics. In: Services HaH, editor.; 2010.

8. Chiu CJ, Taylor A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. Prog Retin Eye Res. 2011 Jan;30:18-53.

9. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr. 2002 Jul;76:274S-80S.

10. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981 Mar;34:362-6.

11. Pawlak DB, Kushner JA, Ludwig DS. Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. Lancet. 2004 Aug 28-Sep 3;364:778-85.

12. Scribner KB, Pawlak DB, Ludwig DS. Hepatic steatosis and increased adiposity in mice consuming rapidly vs. slowly absorbed carbohydrate. Obesity (Silver Spring). 2007 Sep;15:2190-9.

13. Scribner KB, Pawlak DB, Aubin CM, Majzoub JA, Ludwig DS. Long-term effects of dietary glycemic index on adiposity, energy metabolism, and physical activity in mice. Am J Physiol Endocrinol Metab. 2008 Nov;295:E1126-31.

14. van Schothorst EM, Bunschoten A, Schrauwen P, Mensink RP, Keijer J. Effects of a high-fat, low- versus high-glycemic index diet: retardation of insulin resistance involves adipose tissue modulation. Faseb J. 2009 Apr;23:1092-101.

15. Isken F, Klaus S, Petzke KJ, Loddenkemper C, Pfeiffer AF, Weickert MO. Impairment of fat oxidation under high vs low glycemic index diet occurs prior to the development of an obese phenotype. Am J Physiol Endocrinol Metab. 2009 Nov 24.

16. Isken F, Weickert MO, Tschop MH, Nogueiras R, Mohlig M, Abdelrahman A, Klaus S, Thorens B, Pfeiffer AF. Metabolic effects of diets differing in

glycaemic index depend on age and endogenous glucose-dependent insulinotrophic polypeptide in mice. Diabetologia. 2009 Oct;52:2159-68.

17. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001 Feb;44:129-46.

18. Chiarelli F, de Martino M, Mezzetti A, Catino M, Morgese G, Cuccurullo F, Verrotti A. Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. J Pediatr. 1999 Apr;134:486-91.

19. Kostolanska J, Jakus V, Barak L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2009 May;22:433-42.

20. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. J Clin Invest. 1997 Feb 1;99:457-68.

21. Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, Koike T, Wakayama I, Yanagihara R, et al. Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol. 1998 Oct;153:1149-55.

22. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. Proc Natl Acad Sci U S A. 1994 Nov 22;91:11704-8.

23. Haitoglou CS, Tsilibary EC, Brownlee M, Charonis AS. Altered cellular interactions between endothelial cells and nonenzymatically glucosylated laminin/type IV collagen. J Biol Chem. 1992 Jun 25;267:12404-7.

24. Busch M, Franke S, Ruster C, Wolf G. Advanced glycation end-products and the kidney. Eur J Clin Invest. 2010 Aug;40:742-55.

25. Ruster C, Bondeva T, Franke S, Forster M, Wolf G. Advanced glycation end-products induce cell cycle arrest and hypertrophy in podocytes. Nephrol Dial Transplant. 2008 Jul;23:2179-91.

26. Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Hanssen KF, Laakso M. Increased serum levels of methylglyoxal-derived hydroimidazolone-AGE are associated with increased cardiovascular disease mortality in nondiabetic women. Atherosclerosis. 2009 Aug;205:590-4.

27. Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, Hanssen KF. Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. Diabetes Care. 1999 Jul;22:1186-90.

28. van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJ, Schalkwijk CG, Bronzwaer JG, et al. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. Circulation. 2008 Jan 1;117:43-51.

29. Ma H, Li SY, Xu P, Babcock SA, Dolence EK, Brownlee M, Li J, Ren J. Advanced glycation endproduct (AGE) accumulation and AGE receptor (RAGE)

up-regulation contribute to the onset of diabetic cardiomyopathy. J Cell Mol Med. 2009 Aug;13:1751-64.

30. Rojas A, Mercadal E, Figueroa H, Morales MA. Advanced Glycation and ROS: a link between diabetes and heart failure. Curr Vasc Pharmacol. 2008 Jan;6:44-51.

31. Celec P, Hodosy J, Jani P, Janega P, Kudela M, Kalousova M, Holzerova J, Parrak V, Halcak L, et al. Advanced glycation end products in myocardial reperfusion injury. Heart Vessels. 2010 May 12.

32. Dei R, Takeda A, Niwa H, Li M, Nakagomi Y, Watanabe M, Inagaki T, Washimi Y, Yasuda Y, et al. Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease. Acta Neuropathol. 2002 Aug;104:113-22.

33. Jono T, Kimura T, Takamatsu J, Nagai R, Miyazaki K, Yuzuriha T, Kitamura T, Horiuchi S. Accumulation of imidazolone, pentosidine and N(epsilon)-(carboxymethyl)lysine in hippocampal CA4 pyramidal neurons of aged human brain. Pathol Int. 2002 Sep;52:563-71.

34. Girones X, Guimera A, Cruz-Sanchez CZ, Ortega A, Sasaki N, Makita Z, Lafuente JV, Kalaria R, Cruz-Sanchez FF. N epsilon-carboxymethyllysine in brain aging, diabetes mellitus, and Alzheimer's disease. Free Radic Biol Med. 2004 May 15;36:1241-7.

35. Shang F, Gong X, Taylor A. Activity of ubiquitin-dependent pathway in response to oxidative stress. Ubiquitin-activating enzyme is transiently up-regulated. J Biol Chem. 1997;272:23086-93.

36. Shang F, Nowell TR, Jr., Taylor A. Removal of oxidatively damaged proteins from lens cells by the ubiquitin-proteasome pathway. Exp Eye Res. 2001 Aug;73:229-38.

37. Pickart CM, Eddins MJ. Ubiquitin: structures, functions, mechanisms. Biochim Biophys Acta. 2004 Nov 29;1695:55-72.

38. Munch G, Thome J, Foley P, Schinzel R, Riederer P. Advanced glycation endproducts in ageing and Alzheimer's disease. Brain Res Brain Res Rev. 1997 Feb;23:134-43.

39. Moreira PI, Sayre LM, Zhu X, Nunomura A, Smith MA, Perry G. Detection and localization of markers of oxidative stress by in situ methods: application in the study of Alzheimer disease. Methods Mol Biol.610:419-34.

40. Wu M, Bian Q, Liu Y, Fernandes AF, Taylor A, Pereira P, Shang F. Sustained oxidative stress inhibits NF-kappaB activation partially via inactivating the proteasome. Free Radic Biol Med. 2009 Jan 1;46:62-9.

41. Zhang X, Gaspard JP, Mizukami Y, Li J, Graeme-Cook F, Chung DC. Overexpression of cyclin D1 in pancreatic beta-cells in vivo results in islet hyperplasia without hypoglycemia. Diabetes. 2005 Mar;54:712-9.

42. Ader M, Bergman RN. Insulin sensitivity in the intact organism. Baillieres Clin Endocrinol Metab. 1987 Nov;1:879-910.

43. Uribarri J, Cai W, Ramdas M, Goodman S, Pyzik R, Chen X, Zhu L, Striker GE, Vlassara H. Restriction of Advanced Glycation End Products Improves Insulin Resistance in Human Type 2 Diabetes: Potential role of AGER1 and SIRT1. Diabetes Care. 2011 Jul;34:1610-6.

44. Syed IA. Glycated haemoglobin; past, present, and future are we ready for the change. J Pak Med Assoc. 2011 Apr;61:383-8.

45. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology. 2005 Jul;15:16R-28R.

46. Yau JL, McNair KM, Noble J, Brownstein D, Hibberd C, Morton N, Mullins JJ, Morris RG, Cobb S, Seckl JR. Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-hydroxysteroid dehydrogenase type 1 knock-out mice. J Neurosci. 2007 Sep 26;27:10487-96.

47. Cai W, He JC, Zhu L, Chen X, Zheng F, Striker GE, Vlassara H. Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. Am J Pathol. 2008 Aug;173:327-36.

48. Taguchi A, Wartschow LM, White MF. Brain IRS2 signaling coordinates life span and nutrient homeostasis. Science. 2007 Jul 20;317:369-72.

49. Rabbani N, Thornalley PJ. Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino Acids. 2010 Oct 21.

50. Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med (Berl). 2003 Nov;81:678-99.

51. Miyata T, Inagi R, K. A, Yamada Y, Horie K, Sakai H, Uchida K, Kurokawa K. Generation of protein carbonyls by glycoxidation and lipoxidation reactions

with autoxidation products of ascorbic acid and polyunsaturated fatty acids. Federation of European Biology and Science Letters. 1998;427:24-8.

52. Ferland A, Brassard P, Lemieux S, Bergeron J, Bogaty P, Bertrand F, Simard S, Poirier P. Impact of high-fat /low-carbohydrate, high-, low-glycaemic index or low-caloric meals on glucose regulation during aerobic exercise in Type 2 diabetes. Diabet Med. 2009 Jun;26:589-95.

53. Chiu C-J, Liu, S., Willett, W. C., Wolever, T.M.S., Brand-Miller, J. C. Barclay, A. C., A. Taylor. Informing food choices and health outcomes by use of the dietary glycemic index Nutrition Reviews. 2011 April;69:231-42.

54. Wu J, Zhang R, Torreggiani M, Ting A, Xiong H, Striker GE, Vlassara H, Zheng F. Induction of diabetes in aged C57B6 mice results in severe nephropathy: an association with oxidative stress, endoplasmic reticulum stress, and inflammation. Am J Pathol. 2010 May;176:2163-76.

55. Yokota T, Kinugawa S, Hirabayashi K, Matsushima S, Inoue N, Ohta Y, Hamaguchi S, Sobirin MA, Ono T, et al. Oxidative stress in skeletal muscle impairs mitochondrial respiration and limits exercise capacity in type 2 diabetic mice. Am J Physiol Heart Circ Physiol. 2009 Sep;297:H1069-77.

56. So PW, Yu WS, Kuo YT, Wasserfall C, Goldstone AP, Bell JD, Frost G. Impact of resistant starch on body fat patterning and central appetite regulation. PLoS One. 2007;2:e1309.

57. Le Leu RK, Hu Y, Brown IL, Young GP. Effect of high amylose maize starches on colonic fermentation and apoptotic response to DNA-damage in the colon of rats. Nutr Metab (Lond). 2009;6:11.

58. Regmi PR, van Kempen TA, Matte JJ, Zijlstra RT. Starch with high amylose and low in vitro digestibility increases short-chain fatty acid absorption, reduces peak insulin secretion, and modulates incretin secretion in pigs. J Nutr. 2011 Mar;141:398-405.

59. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr. 2005 Sep;82:559-67.

60. Barth RJ. Insulin resistance, obesity and the metabolic syndrome. S D Med. 2011;Spec No:22-7.

61. Adams MK, Simpson JA, Aung KZ, Makeyeva GA, Giles GG, English DR, Hopper J, Guymer RH, Baird PN, Robman LD. Abdominal obesity and age-related macular degeneration. Am J Epidemiol. 2011 Jun 1;173:1246-55.

62. Azagury DE, Lautz DB. Obesity overview: epidemiology, health and financial impact, and guidelines for qualification for surgical therapy. Gastrointest Endosc Clin N Am. 2011 Apr;21:189-201.

63. Ahmed MU, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW. N-epsilon-(carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem J. 1997 Jun 1;324 (Pt 2):565-70.

64. Goligorsky MS, Chen J, Patschan S. Stress-induced premature senescence of endothelial cells: a perilous state between recovery and point of no return. Curr Opin Hematol. 2009 May;16:215-9.

65. Guix FX, Ill-Raga G, Bravo R, Nakaya T, de Fabritiis G, Coma M, Miscione GP, Villa-Freixa J, Suzuki T, et al. Amyloid-dependent triosephosphate isomerase nitrotyrosination induces glycation and tau fibrillation. Brain. 2009 May;132:1335-45.

66. Vlassara H, Uribarri J, Ferrucci L, Cai W, Torreggiani M, Post JB, Zheng F, Striker GE. Identifying advanced glycation end products as a major source of oxidants in aging: implications for the management and/or prevention of reduced renal function in elderly persons. Semin Nephrol. 2009 Nov;29:594-603.

67. Wei Y, Chen L, Chen J, Ge L, He RQ. Rapid glycation with D-ribose induces globular amyloid-like aggregations of BSA with high cytotoxicity to SH-SY5Y cells. BMC Cell Biol. 2009;10:10.

68. Shang F, Taylor A. Ubiquitin-proteasome pathway and cellular responses to oxidative stress. Free Radic Biol Med. 2011 Apr 8.

4.7 Figure Legends

Figure 1. Study design composed of two age groups of C57BL/6 mice. Arrows indicate blood drawing throughout the study. After the mice were killed, tissues were harvested and processed for biochemical analysis.

Figure 2. Metabolic profile of 17-month-old C57BL/6 mice pair-fed a high or low GI diet. Mice were fully acclimated onto a high or low GI diet by 6.5 months of age and were fed until 17 months of age. **A**) Body weight of mice in the high GI (solid line) and low GI (dotted line) groups. **B**) Feed-deprived blood glucose

was measured in the high GI (solid line) and low GI (dotted line) groups. **C**) Area under the curve (AUC) for blood glucose during intraperitoneal oral glucose tolerance tests administered to high GI (black bars) and low GI (gray bars) groups. **D**) Changes from baseline blood glucose levels following injection of insulin during intraperitoneal insulin tolerance tests at 14 (top panel) and 17 (bottom panel) months of age (after 33 and 46 weeks of experimental diets, respectively). Solid line represents the high GI group and dotted line represents the low GI group. **E**) Feed-deprived insulin levels in 17-month-old mice in the high GI (black bar) and low GI (gray bar) groups. **F**) Homeostatic assessment of insulin resistance (HOMA-IR) values for the high GI (black bar) and low GI (gray bar) groups at 17 months of age. **G**) Levels of glycated hemoglobin in 17-month-old mice fed high GI (black bar) or low GI (gray bar) diets. For all graphs, error bars represent SEM. * indicates a difference between diet groups, $p < 0.05$.

Figure 3. Metabolic profile of 23.5-month-old C57BL/6 mice pair-fed a high or low GI diet. Mice were fully acclimated onto a high or low GI diet by 17.5 months of age and were fed until 23.5 months of age. **A**) Body weight of mice in the high GI (solid line) and low GI (dotted line) groups. **B**) Feed-deprived blood glucose in the high GI (solid line) and low GI (dotted line) groups. **C**) Area under the curve (AUC) for blood glucose during intraperitoneal oral glucose tolerance tests administered to high GI (black bars) and low GI (gray bars) groups. **D**) Feed-deprived insulin levels of 23.5-month-old mice in the high GI (black bar) or low GI (gray bar) groups. **E**) Homeostatic assessment of insulin resistance

(HOMA-IR) values for the high GI (black bar) and low GI (gray bar) groups at 23.5 months of age. For all graphs, error bars represent SEM. * indicates a difference between diet groups, $p < 0.05$.

Figure 4. Glycative stress in tissues from 23.5-month-old mice fed high or low GI diets. Accumulation of MG-H1-modified protein was measured by Western blot in kidneys (**A**), hearts (**C**) and brains (**E**). "H" indicates a sample from the high GI group (n=4), and "L" indicates a sample from the low GI group (n=6). Ponceau S staining was used as a loading control. Blots shown are representative of the entire sample. Densitometric accumulation of MG-H1-modified protein in kidneys, hearts and brains relative to Ponceau staining is quantified in panels **B, D,** and **F** respectively, for all of the samples in each diet group. Black bars represent samples in the high GI group and gray bars represent samples in the low GI group. * indicates a difference between diet groups, $p = 0.05$. Error bars represent SEM.

Figure 5. Oxidative stress in tissues from 17-month-old mice fed high or low GI diets. Accumulation of protein carbonyls (**A, C**) was measured by Western blot in kidneys (**A**) and brains (**C**). "H" indicates a sample from the high GI group (n=9) and "L" indicates a sample from the low GI group (n=8). Dinitrophenyl Hydrazine (DNP) antibody was used to detect protein carbonyls. Ponceau S staining (**A**) and R250 (**C**) were used as loading controls. Blots shown are representative of the entire sample. Densitometric accumulation of

carbonylated protein in kidneys and brains relative to loading control is quantified in panels **B** and **D** respectively, for all of the samples in each diet group. Black bars represent samples in the high GI group, and gray bars represent samples in the low GI group. * indicates a differences between diet groups, $p < 0.05$. Error bars represent SEM.

Figure 6. Proteasome activity in heart, kidney and brain from 23.5-month-old mice fed high or low GI diets. Proteasome activity was measured in the hearts (**A**), kidneys (**B**) and brains (**C**) of 23.5 month old mice fed a high GI (black bar; n=4) or a low GI (gray bar; n=6) diet. * indicates a difference between diet groups, $p < 0.05$. Error bars represent SEM.

4.8 Tables

Table 1. Diet Composition.

Ingredient	High GI Diet (g/kg)	Low GI Diet (g/kg)
100% amylopectin (Amioca starch)	534	0
70% amylose/30% amylopectin (Hylon VII starch)	0	534
Casein	200	200
Sucrose	85	85
Soybean Oil	56	56
Wheat Bran	50	50
DL Methionine	2	2
Vitamin Mix	10	10
Mineral Mix	35	35
Hydroquinone	8	8
Macronutrient % of Total Energy		
Carbohydrate	65	65
Protein	21	21
Fat	14	14

4.9 Figures

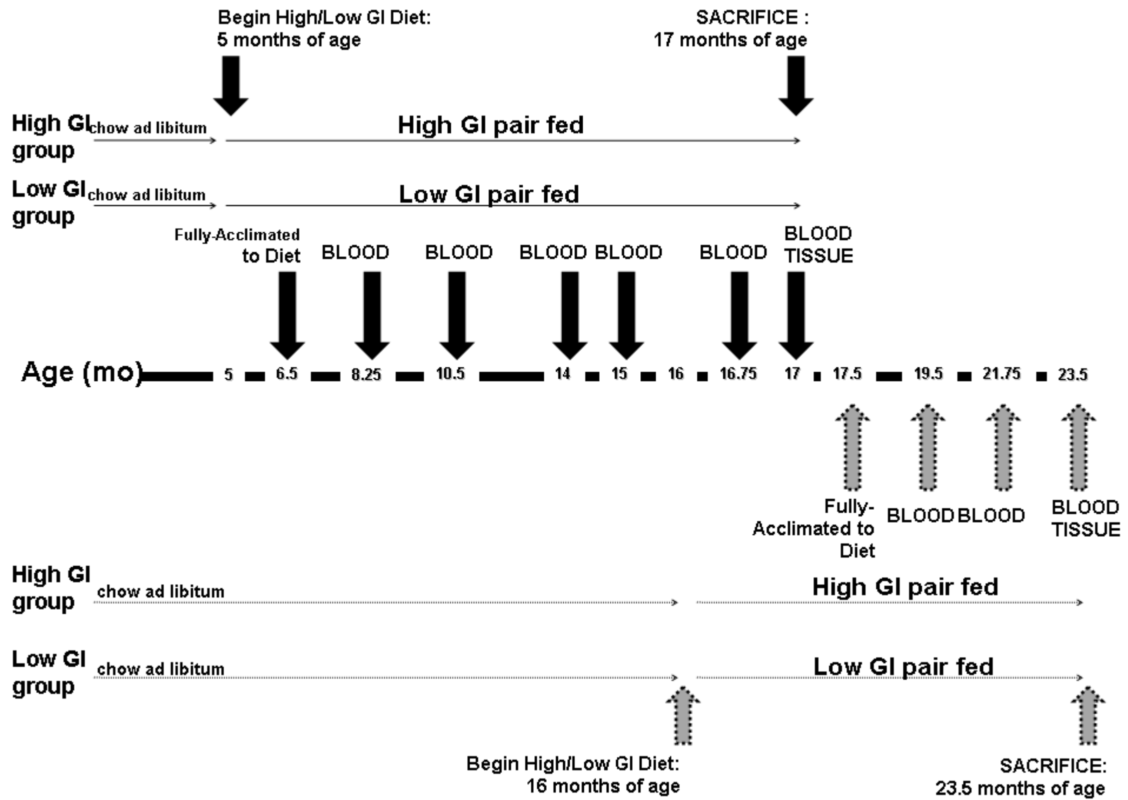
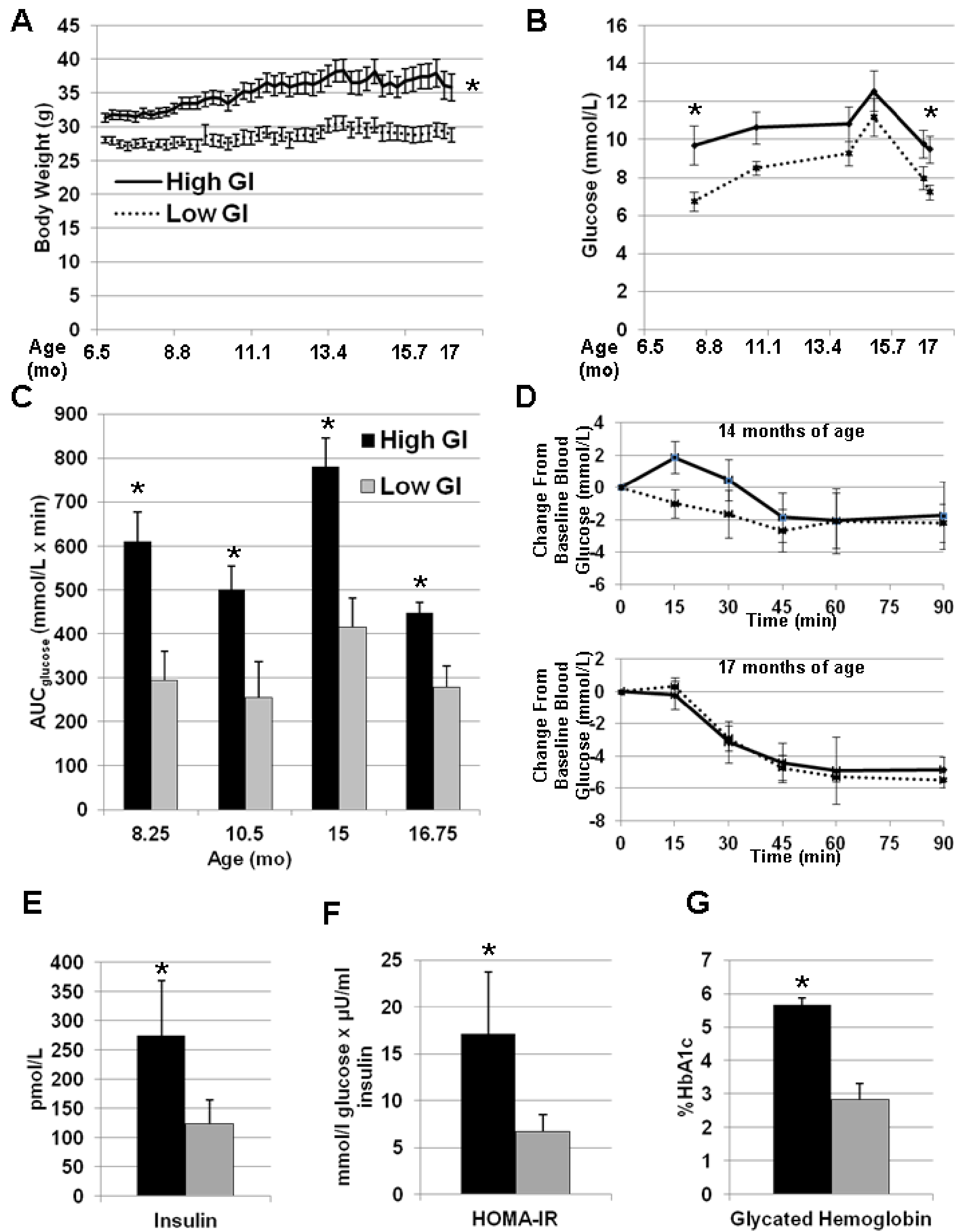


Figure 1.



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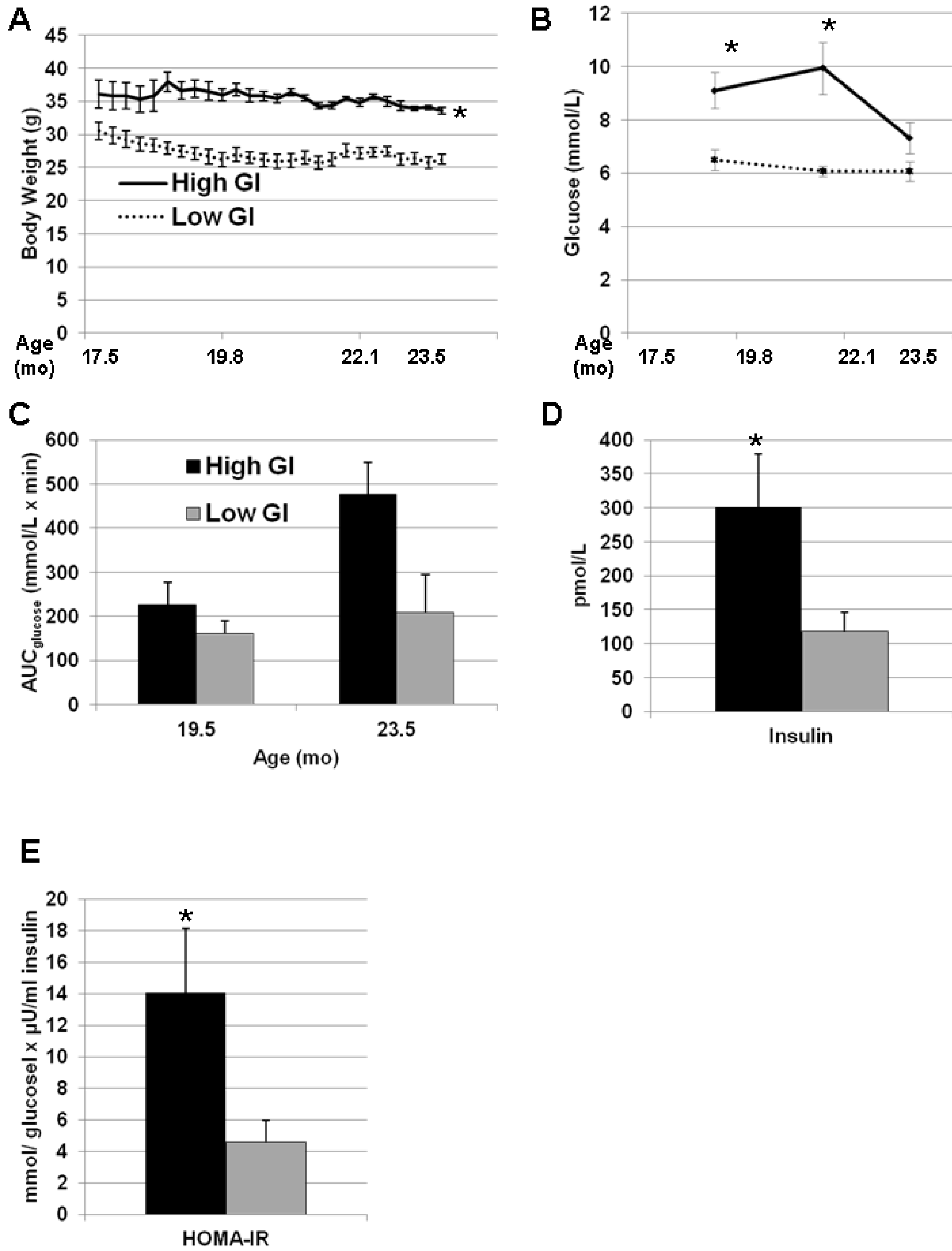

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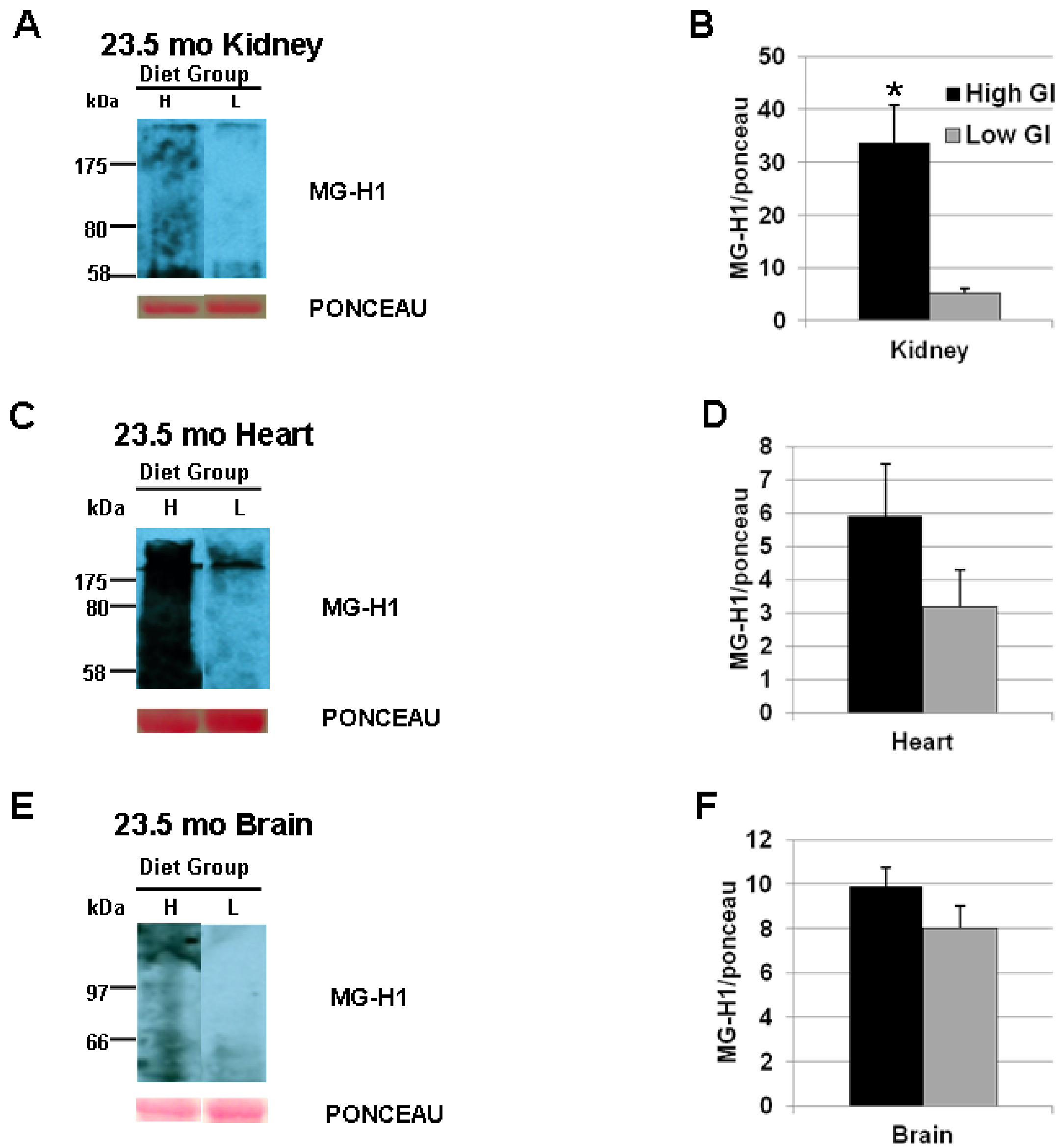

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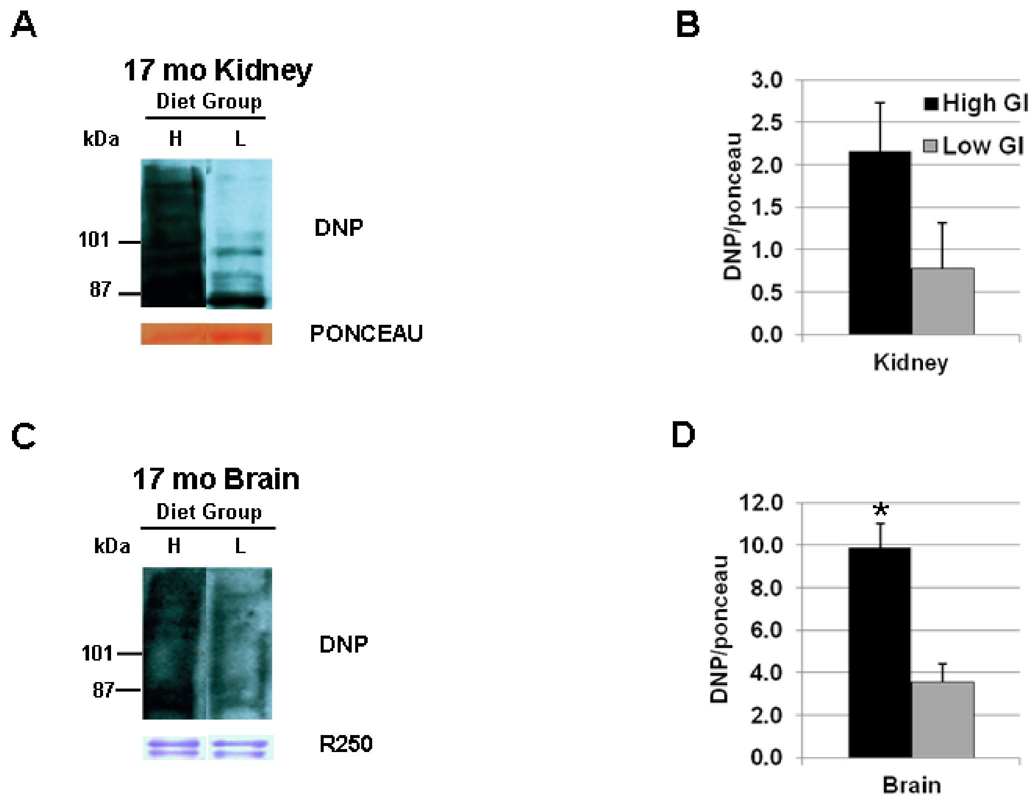


Figure 5.

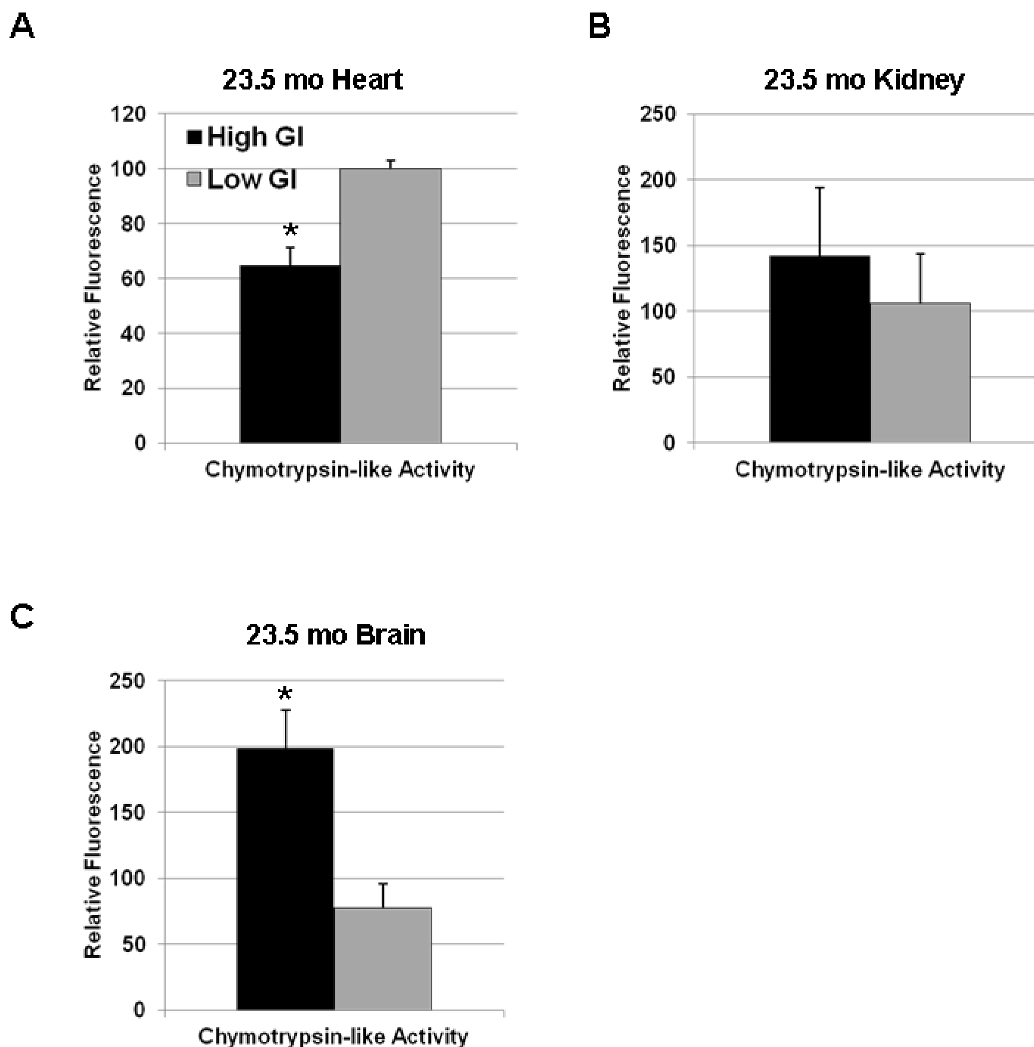


Figure 6.

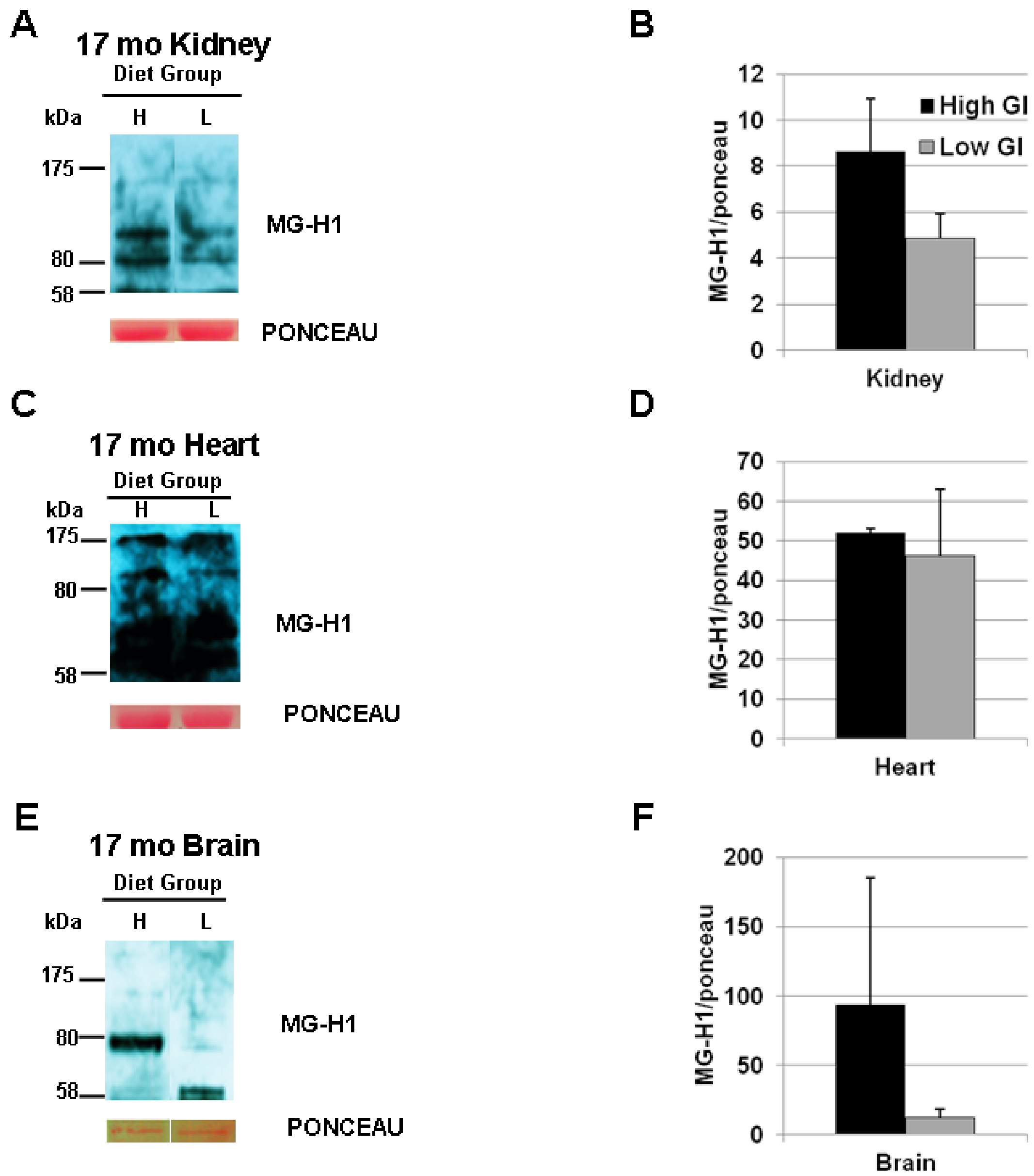
4.10 Supplementary Material
Figure Legends

Supplementary Figure S1. Glycative stress in tissues from 17-month-old mice fed high or low GI diets. Accumulation of MG-H1-modified protein was measured by Western blot in kidneys (**A**), hearts (**C**) and brains (**E**). "H" indicates a sample from the high GI group (n=9), and "L" indicates a sample from the low GI group (n=8). Ponceau S staining was used as a loading control. Blots shown are representative of the entire sample. Densitometric accumulation of

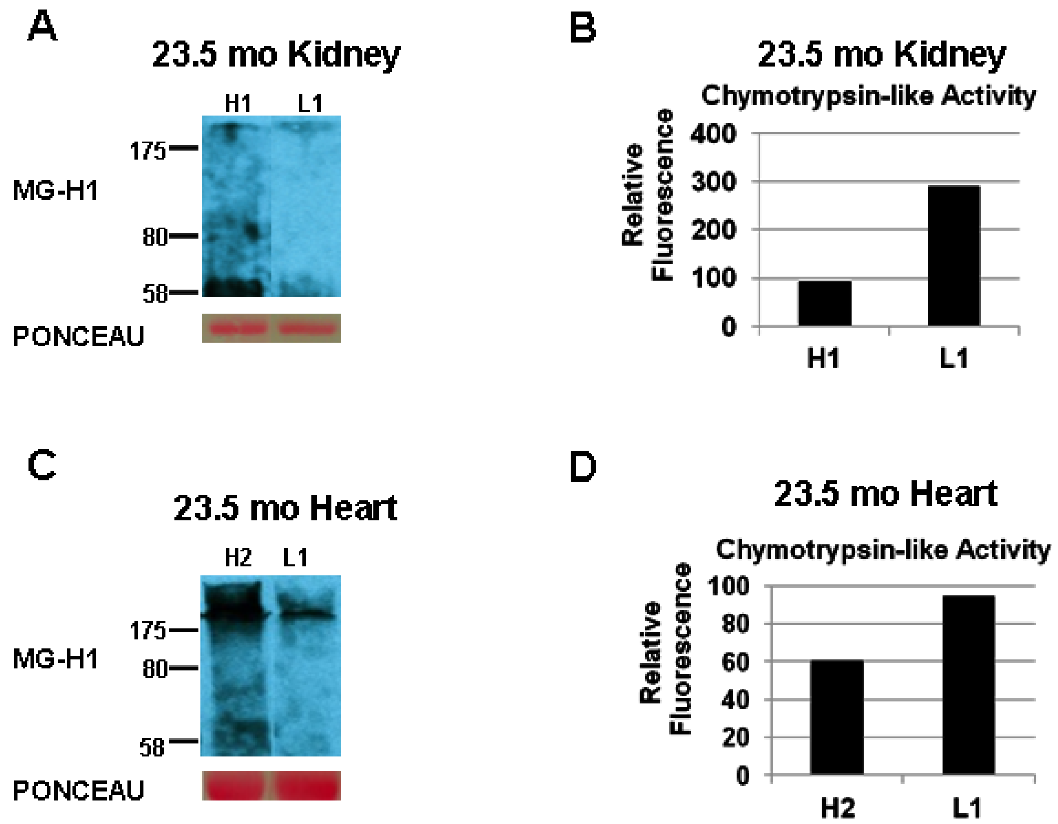
MG-H1-modified protein in kidneys, hearts and brains relative to Ponceau staining is quantified in panels **B, D** and **F** respectively, for all of the samples in each diet group. Black bars represent samples from high GI-fed mice and gray bars represent samples from low GI-fed mice. Error bars represent SEM.

Supplementary Figure S2. AGE accumulation and proteasome activity in the kidney and heart of individual 23.5-month-old mice that consumed a high or low GI diet. MG-H1-modified protein was measured in kidney (**A**) and heart (**C**) using Western blot, with Ponceau S staining as a loading control. Chymotrypsin-like activity of the proteasome was measured using a fluorogenic peptide substrate in kidney (**B**) and heart (**D**). In each panel, samples labeled “H1”, and “H2” were 23.5-month-old mice fed a high GI diet. “L1” was a 23.5-month-old mouse fed a low GI diet.

Figures



Supplementary Figure S1.



Supplementary Figure S2.

Chapter 5. Summary and Discussion

In this work, we used two sets of C57BL/6 mice to corroborate human epidemiological findings that indicate that consuming higher glycemic index (GI) diets is associated with increased risk for early AMD (1-4). We found that consumption of a higher GI diet increased the appearance of AMD-like lesions and increased accumulation of advanced glycation end products (AGEs) in the retina. In testing our hypothesis we also established that high GI diets induce outer retinal pathology in C57BL/6 mice that is associated with AMD-like lesions. This intervention can be used as a platform on which to test drugs that may delay this disease.

To determine if the relationship between dietary GI and AGE accumulation in tissues is systemic (and therefore begin to explain the role of GI in risk for diseases such as kidney disease, cardiovascular disease and neurodegenerative diseases), we also measured AGE accumulation in the kidneys, hearts and brains of high and low GI-fed mice (5-7). Consumption of the higher GI diet led to systemic accumulation of AGEs that may be influenced by glycative stress-induced changes in proteasome activity. High GI-fed mice also had impaired glucose tolerance and more insulin resistance relative to the low GI-fed mice. The benefits conferred by the low GI diet in attenuating AGE accumulation were observed more clearly in 23.5- rather than 17-month-old mice. Together, these data suggest that high GI diets exert a systemic stress that impairs glucose clearance and facilitates AGE accumulation in tissue. This toxic accumulation, especially in the context of limited proteolytic capacity, may lead to tissue

damage, as observed by the appearance of AMD-like lesions in retinas that accumulated AGEs.

5.1 Consumption of a Higher GI Diet Increases AMD-Like Lesions

To confirm the association between GI and AMD risk in a biological context, we measured the frequency and severity of AMD-like lesions in middle-aged and older mice, fed high or low GI diets. In each diet group, AMD-like lesions such as basal laminar deposits, cytoplasmic vacuoles and loss of basal infoldings were more prevalent in the 23.5-month-old mice than in the 17-month-old mice (**Chapter 3, Figure 2**). Within each age group, these lesions were also more prevalent in the higher GI-fed mice (**Chapter 3, Figure 2**). In addition to the lesions noted above, photoreceptor abnormalities, loss of nuclei in the inner nuclear layer, and accumulation of AGEs were found in the retinas of higher GI-fed mice (**Chapter 3, Figures 3-5**). Compared to all other diet and age groups, the 23.5-month-old high GI-fed mice exhibited the most AMD-like lesions, supporting epidemiological data linking both age and high GI diets with increased risk for early AMD. In addition, the accumulation of putatively cytotoxic AGEs in higher GI-fed mice suggests that these modified proteins may be etiologic for age-related dysfunction and initiation of AMD pathology.

5.2 Hydroquinone is Not Needed to Elicit AMD-Like Lesions

In our intervention, we used a dietary hydroquinone (HQ) model that was originally developed to investigate the effects of cigarette smoke on AMD (8).

Interestingly, comparison of rates of accumulation of AMD-like lesions between high GI diet-fed mice that were or were not treated with HQ indicated that inclusion of HQ in the diet did not accelerate the appearance of AMD-like lesions. In fact, basal laminar deposits, one of the most commonly used indicators of retinal aging and early AMD, were more prevalent in high GI-fed mice not exposed to HQ, than in high GI-fed mice exposed to HQ (**Chapter 3, Figure 6**) (9). The frequency and severity of many other lesions such as lipofuscin, outer collagenous layer deposits, and cytoplasmic vacuoles were not different between the HQ-fed and non-HQ-fed mice. In addition, HQ did not alter the effects of a high GI diet on glucose clearance, insulin resistance, or levels of glycated hemoglobin (**Chapter 3, Supplementary Figure S1**). Collectively, these data indicate that in the presence of a high GI diet, HQ is not necessary to elicit features of early AMD in mice. Therefore, the high GI-fed C57BL/6 mouse without HQ exposure can be used as a new model of early AMD in which to investigate other preventative interventions. This is a major advantage since HQ is a carcinogen and experiments using HQ require special containment and significantly higher costs.

5.3 Higher GI Diets Impair Glucose Metabolism in Middle-Aged and Older Mice

Assessment of the metabolic changes that accompany GI modulation in older rodents is crucial to understanding how dietary GI may influence risk for chronic diseases such as AMD. Evaluation of glucose and insulin levels in

response to high and low GI diets has only been explored in young animals (1, 10-19). Interestingly, it has been shown that older and younger humans experience different changes in blood glucose levels following consumption of the same food (20). Thus, to begin to understand how high GI diets may increase risk for chronic diseases such as AMD in an age-appropriate model, we measured several parameters of glucose metabolism in 17- and 23.5-month-old mice. Unlike observations in humans, the blood glucose responses to high or low GI diets were similar across age groups in our study. In 17- and 23.5-month-old mice, consistent with reports from young mice, higher GI-fed mice (although not overtly diabetic) exhibited more severe glucose intolerance and insulin resistance (**Chapter 4, Figures 2, 3**) (16). However, the higher GI-fed mice also had significantly greater body weight, which may have contributed to this glucose and insulin-related stress.

The metabolic benefits of a low GI diet compared to typical rodent chow were similar in middle-aged and older mice. The glucose and insulin levels observed in the low GI-fed mice were lower than those observed in similarly aged chow-fed mice (21-25). Collectively, these data indicate that the metabolic benefits of consuming low- rather than high- GI diets extend to middle-aged and older mice as well. Also, the data suggest that low GI diets can help delay age-related impairment of glucose metabolism. Together these findings support the role of lower GI diets in reducing risk for chronic disease.

5.4 High GI Diets Increase Cellular Stress

In our mouse model of early AMD, we observed greater accumulation of AGEs in retinas of high GI-fed mice. These mice also developed more advanced AMD-like lesions more frequently. To determine if glycative stress in other tissues is involved in the association between dietary GI and diseases of those tissues, we measured the accumulation of AGEs in the kidneys, hearts and brains of 17- and 23.5-month-old high GI and low GI-fed mice. Each of these tissues is damaged in a chronic disease that is associated with both high GI diets and AGE accumulation (3, 18, 26-69). We found that consuming the higher GI diets was associated with systemic accumulation of AGEs and protein carbonyls in these tissues, and the most robust effects of GI were observed in the 23.5-month-old mice (**Chapter 4, Figures 4, 5**).

AGEs may accumulate if rates of formation outpace rates of removal. To determine if accumulation of AGEs in these tissues could be due to insufficient proteolytic capacity, we measured proteasome activity in tissues of high and low GI-fed mice. Changes in proteasome activity in response to the higher GI diet were not uniform across all tissues, suggesting that proteasomal activity was probably not rate determining in all tissues with regard to clearance of AGEs (**Chapter 4, Figure 6**). The differences in proteasome activity in response to the high GI diet may be due to different antioxidant activities between these tissues. In rats, it has been shown that the brain and kidney have higher superoxide dismutase and catalase activity compared to the heart (70). As a result, the high GI diet may induce only a mild oxidative stress in the brain and kidney, and a more severe stress in the heart. It has been shown that under conditions of mild

stress (such as in the brains and kidneys of high GI-fed mice), proteasome activity may increase, while more severe stress (such as in the hearts of high GI-fed mice) results in a decrease of proteasome activity (71-74). Our observation of decreased proteasome activity in the heart, and increased proteasome activity in the brain and kidney is consistent with this theory. Furthermore, data from individual mice suggested that AGE accumulation was inversely related to proteasome activity, supporting the hypothesis that AGEs accumulate in certain tissues under conditions of insufficient proteolysis (**Chapter 4, Supplementary Figure S2**).

Collectively, these data suggest that older mice are more susceptible to the intracellular glycoxidative and oxidative stresses associated with consumption of high GI diets. Therefore, future studies investigating the mechanistic link between GI and chronic disease would benefit from the use of old rather than young animals. Furthermore, the association between AGE accumulation and diminished proteasome activity suggests that preservation of ubiquitin-mediated degradation may be one way in which low GI diets protect tissues from oxidative damage.

5.5 Future Research

These data indicate that consumption of a low GI diet limits the accumulation of AGEs and the appearance of AMD-like lesions in the retina, relative to consumption of a high GI diet. The relationship between AGE accumulation and the UPS is less clear, since the response of the proteasome to

a high GI diet varied between tissues. However, there was an inverse association between AGE accumulation and 20S proteasome activity in several tissues, supporting the involvement of the UPS in AGE metabolism.

More research is required to more fully characterize these topics. This exploratory study facilitated the identification of specific AMD-like lesions which were most affected by dietary GI. However, the small sample size was not sufficient to capture the full magnitude of the effect of GI on the appearance of these lesions. Having corroborated epidemiologic data regarding the influence of both age and GI on the appearance of these lesions in our model, it would be appropriate to repeat this study in larger cohorts of high and low GI-fed C57BL/6 mice. The incorporation of HQ into the diets would not be necessary.

In this larger study, food restriction or other measures to maintain equivalent body weights between treatment groups should be used. Finding similar results in larger studies would establish that the differing retinal phenotypes are due to different dietary GI, rather than the metabolic stresses associated with increased body weight. Although body weight did not appear to correlate with the appearance of any of the retinal lesions in this pilot study, body weight differences may have influenced systemic stress levels.

Inflammatory and oxidative stress pathways have both been implicated in AMD pathogenesis. Our data suggest that oxidative stress plays a significant role in the appearance of AMD-like lesions in our model, as demonstrated by the deposition of AGEs in the retina. We did not observe GI-related changes in inflammatory markers such as C5b-9 accumulation in the retina, or CRP in the

plasma. However, there are many other inflammatory mediators that may be influenced by GI to modulate risk for AMD. In the future, retinas of mice fed high and low GI diets should also be analyzed for deposition of other complement pathway proteins, recruitment of macrophages and activation of microglia.

Characterization of how UPS activity is associated with AGE accumulation in the retina in the context of diets of different GI warrants further investigation as well. High GI-induced changes in 26S (rather than 20S) proteasome activity should be measured (using ornithine decarboxylase as a substrate), along with the effects of a high GI diet and AGE accumulation on ubiquitin conjugate formation and degradation of UPS substrates. Understanding how diet and AGEs influence UPS activity may help explain additional ways in which glycative stress and AGEs are involved in AMD pathogenesis. Such findings would complement mechanistic data from RPE cell culture and *in vitro* studies (Uchiki et al, 2011, *in press*). These studies have shown that glycative stress impairs UPS-mediated degradation of substrates by inhibiting ubiquitin conjugate formation and activity of ubiquitin conjugating enzymes (Uchiki et al, 2011, *in press*). Inhibition of the UPS through glycative stress may promote degradation of substrates by other cellular protein quality control pathways such as through the lysosome (Uchiki et al, 2011, *in press*).

The assessment of each of these parameters (appearance of AMD-like lesions, inflammatory markers, UPS activity) would ideally be measured serially in mice over the duration of the experimental feeding. This chronological study

would not only provide insight into the molecular development of early AMD, but would indicate how AGEs and the UPS contribute to its progression.

The data presented here indicate that dietary GI can modulate AGE accumulation in tissues (other than the eye) that are subject to chronic diseases characterized by accumulation of AGEs. Thus, similar studies are warranted to begin to elucidate mechanisms that relate consuming diets with different GIs on AGE accumulation and pathology in heart, kidney and neuronal tissue.

5.6 Implications for Public Policy

Verification of the relationship between dietary GI and disease in other model systems would further support the need for long-term human intervention studies to explore the benefits of lower GI diets on the age-related diseases noted above. The results of such studies would greatly influence public health policy because intervention trials are considered to be the "gold standard." At a time when the proportion of our population that is elderly is growing rapidly and the health care budget is shrinking, more effective preventative health is urgently needed (75, 76). Positive outcomes of such studies can be anticipated because drugs which simulated the effects of consuming lower GI diets were shown to delay the onset of diabetes and cardiovascular disease (CVD) (7, 10, 77-81). In addition to the elderly, diabetes, insulin resistance, and CVD are more common in obese individuals, which now represent about 34% of the American population (82, 83). These conditions are also characterized by high levels of AGEs (1, 2, 10, 18, 19, 28-31, 79, 80, 84, 85). Consumption of low GI diets for the purpose

of attenuating AGE accumulation may therefore significantly improve health prospects not only for the elderly, but especially for this overweight population (86). Changing to lower GI diets would be relatively inexpensive and would dramatically reduce the financial and emotional burden of chronic disease in high-risk individuals such as the obese, and elderly.

5.7 References

1. Chiu CJ, Hubbard LD, Armstrong J, Rogers G, Jacques PF, Chylack J, L. T., Hankinson SE, Willett WC, Taylor A. Dietary glycemic index and carbohydrate in relation to early age-related macular degeneration. Am J Clin Nutr. 2006;83:880-6.

2. Chiu CJ, Milton RC, Klein R, Gensler G, Taylor A. Dietary carbohydrate and the progression of age-related macular degeneration: a prospective study from the Age-Related Eye Disease Study. Am J Clin Nutr. 2007 Oct;86:1210-8.

3. Chiu CJ, Milton RC, Klein R, Gensler G, Taylor A. Dietary Glycemic Index Is Related to Progression of Age-Related Macular Degeneration. Investigative Ophthalmology & Visual Science. 2007;48:2101.

4. Kaushik S, Wang JJ, Flood V, Tan JS, Barclay AW, Wong TY, Brand-Miller J, Mitchell P. Dietary glycemic index and the risk of age-related macular degeneration. Am J Clin Nutr. 2008 Oct;88:1104-10.

5. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001 Feb;44:129-46.

6. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. J Clin Invest. 1997 Feb 1;99:457-68.

7. Chiarelli F, de Martino M, Mezzetti A, Catino M, Morgese G, Cuccurullo F, Verrotti A. Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. J Pediatr. 1999 Apr;134:486-91.

8. Espinosa-Heidmann DG, Suner IJ, Catanuto P, Hernandez EP, Marin-Castano ME, Cousins SW. Cigarette Smoke-Related Oxidants and the Development of Sub-RPE Deposits in an Experimental Animal Model of Dry AMD. Invest Ophthalmol Vis Sci. 2006 Feb;47:729-37.

9. Sarks S, Cherepanoff S, Killingsworth M, Sarks J. Relationship of Basal laminar deposit and membranous debris to the clinical presentation of early age-related macular degeneration. Invest Ophthalmol Vis Sci. 2007 Mar;48:968-77.

10. Jenkins DJ, Kendall CW, McKeown-Eyssen G, Josse RG, Silverberg J, Booth GL, Vidgen E, Josse AR, Nguyen TH, et al. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. Jama. 2008 Dec 17;300:2742-53.

11. Pawlak DB, Kushner JA, Ludwig DS. Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. Lancet. 2004 Aug 28-Sep 3;364:778-85.

12. Scribner KB, Pawlak DB, Ludwig DS. Hepatic steatosis and increased adiposity in mice consuming rapidly vs. slowly absorbed carbohydrate. Obesity (Silver Spring). 2007 Sep;15:2190-9.

13. Scribner KB, Pawlak DB, Aubin CM, Majzoub JA, Ludwig DS. Long-term effects of dietary glycemic index on adiposity, energy metabolism, and physical activity in mice. Am J Physiol Endocrinol Metab. 2008 Nov;295:E1126-31.

14. van Schothorst EM, Bunschoten A, Schrauwen P, Mensink RP, Keijer J. Effects of a high-fat, low- versus high-glycemic index diet: retardation of insulin resistance involves adipose tissue modulation. Faseb J. 2009 Apr;23:1092-101.

15. Isken F, Klaus S, Petzke KJ, Loddenkemper C, Pfeiffer AF, Weickert MO. Impairment of fat oxidation under high vs low glycemic index diet occurs prior to the development of an obese phenotype. Am J Physiol Endocrinol Metab. 2009 Nov 24.

16. Isken F, Weickert MO, Tschop MH, Nogueiras R, Mohlig M, Abdelrahman A, Klaus S, Thorens B, Pfeiffer AF. Metabolic effects of diets differing in glycaemic index depend on age and endogenous glucose-dependent insulinotrophic polypeptide in mice. Diabetologia. 2009 Oct;52:2159-68.

17. Wolever TM, Jenkins DJ, Vuksan V, Jenkins AL, Wong GS, Josse RG. Beneficial effect of low-glycemic index diet in overweight NIDDM subjects. Diabetes Care. 1992 Apr;15:562-4.

18. McKeown NM, Meigs JB, Liu S, Rogers G, Yoshida M, Saltzman E, Jacques PF. Dietary carbohydrates and cardiovascular disease risk factors in the Framingham offspring cohort. J Am Coll Nutr. 2009 Apr;28:150-8.

19. Chiu CJ, Milton RC, Gensler G, Taylor A. Association between dietary glycemic index and age-related macular degeneration in nondiabetic participants in the Age-Related Eye Disease Study. Am J Clin Nutr. 2007 Jul;86:180-8.

20. Basu R, Dalla Man C, Campioni M, Basu A, Klee G, Toffolo G, Cobelli C, Rizza RA. Effects of age and sex on postprandial glucose metabolism: differences in glucose turnover, insulin secretion, insulin action, and hepatic insulin extraction. Diabetes. 2006 Jul;55:2001-14.

21. Zhang X, Gaspard JP, Mizukami Y, Li J, Graeme-Cook F, Chung DC. Overexpression of cyclin D1 in pancreatic beta-cells in vivo results in islet hyperplasia without hypoglycemia. Diabetes. 2005 Mar;54:712-9.

22. Taguchi A, Wartschow LM, White MF. Brain IRS2 signaling coordinates life span and nutrient homeostasis. Science. 2007 Jul 20;317:369-72.

23. Cai W, He JC, Zhu L, Chen X, Zheng F, Striker GE, Vlassara H. Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. Am J Pathol. 2008 Aug;173:327-36.

24. Yau JL, McNair KM, Noble J, Brownstein D, Hibberd C, Morton N, Mullins JJ, Morris RG, Cobb S, Seckl JR. Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-hydroxysteroid dehydrogenase type 1 knock-out mice. J Neurosci. 2007 Sep 26;27:10487-96.

25. Lee S, Muniyappa R, Yan X, Chen H, Yue LQ, Hong EG, Kim JK, Quon MJ. Comparison between surrogate indexes of insulin sensitivity and resistance and hyperinsulinemic euglycemic clamp estimates in mice. Am J Physiol Endocrinol Metab. 2008 Feb;294:E261-70.

26. Brand-Miller J, McMillan-Price J, Steinbeck K, Caterson I. Dietary glycemic index: health implications. J Am Coll Nutr. 2009 Aug;28 Suppl:446S-9S.

27. Murakami K, Miyake Y, Sasaki S, Tanaka K, Fukushima W, Kiyohara C, Tsuboi Y, Yamada T, Oeda T, et al. Dietary glycemic index is inversely associated with the risk of Parkinson's disease: a case-control study in Japan. Nutrition. 2010 May;26:515-21.

28. Semba RD, Fink JC, Sun K, Bandinelli S, Guralnik JM, Ferrucci L. Carboxymethyl-lysine, an advanced glycation end product, and decline of renal function in older community-dwelling adults. Eur J Nutr. 2009 Feb;48:38-44.

29. Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH, Willett WC. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr. 1999;70:412-9.

30. Riegersperger M, Sunder-Plassmann G. How to prevent progression to end stage renal disease. J Ren Care. 2007 Jul-Sep;33:105-7.

31. Gopinath B, Harris DC, Flood VM, Burlutsky G, Brand-Miller J, Mitchell P. Carbohydrate nutrition is associated with the 5-year incidence of chronic kidney disease. J Nutr. 2011 Mar;141:433-9.

32. Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des. 2005;11:2279-99.

33. Busch M, Franke S, Ruster C, Wolf G. Advanced glycation end-products and the kidney. Eur J Clin Invest. 2010 Aug;40:742-55.

34. Matsuyama Y, Terawaki H, Terada T, Era S. Albumin thiol oxidation and serum protein carbonyl formation are progressively enhanced with advancing stages of chronic kidney disease. Clin Exp Nephrol. 2009 Aug;13:308-15.

35. Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. Kidney Int. 1999 Feb;55:389-99.

36. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovasc Res. 1998 Mar;37:586-600.

37. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA, Cerami A, Vlassara H. Advanced glycosylation end products in patients with diabetic nephropathy. N Engl J Med. 1991 Sep 19;325:836-42.

38. Ruster C, Bondeva T, Franke S, Forster M, Wolf G. Advanced glycation end-products induce cell cycle arrest and hypertrophy in podocytes. Nephrol Dial Transplant. 2008 Jul;23:2179-91.

39. Oya T, Hattori N, Mizuno Y, Miyata S, Maeda S, Osawa T, Uchida K. Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts. J Biol Chem. 1999 Jun 25;274:18492-502.

40. Chougale AD, Bhat SP, Bhujbal SV, Zambare MR, Puntambekar S, Somani RS, Boppana R, Giri AP, Kulkarni MJ. Proteomic Analysis of Glycated Proteins from Streptozotocin-Induced Diabetic Rat Kidney. Mol Biotechnol. 2011 Apr 23.

41. Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Hanssen KF, Laakso M. Increased serum levels of methylglyoxal-derived hydroimidazolone-AGE are associated with increased cardiovascular disease mortality in nondiabetic women. Atherosclerosis. 2009 Aug;205:590-4.

42. Kilhovd BK, Berg TJ, Birkeland KI, Thorsby P, Hanssen KF. Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. Diabetes Care. 1999 Sep;22:1543-8.

43. Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, Hanssen KF. Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. Diabetes Care. 1999 Jul;22:1186-90.

44. van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJ, Schalkwijk CG, Bronzwaer JG, et al. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. Circulation. 2008 Jan 1;117:43-51.

45. Ma H, Li SY, Xu P, Babcock SA, Dolence EK, Brownlee M, Li J, Ren J. Advanced glycation endproduct (AGE) accumulation and AGE receptor (RAGE) up-regulation contribute to the onset of diabetic cardiomyopathy. J Cell Mol Med. 2009 Aug;13:1751-64.

46. Rojas A, Mercadal E, Figueroa H, Morales MA. Advanced Glycation and ROS: a link between diabetes and heart failure. Curr Vasc Pharmacol. 2008 Jan;6:44-51.

47. Celec P, Hodosy J, Jani P, Janega P, Kudela M, Kalousova M, Holzerova J, Parrak V, Halcak L, et al. Advanced glycation end products in myocardial reperfusion injury. Heart Vessels. 2010 May 12.

48. Simm A, Casselmann C, Schubert A, Hofmann S, Reimann A, Silber RE. Age associated changes of AGE-receptor expression: RAGE upregulation is associated with human heart dysfunction. Exp Gerontol. 2004 Mar;39:407-13.

49. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology. 2005 Jul;15:16R-28R.

50. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001;108:949-55.

51. Schmidt AM, Yan SD, Brett J, Mora R, Nowygrod R, Stern D. Regulation of human mononuclear phagocyte migration by cell surface-binding proteins for advanced glycation end products. J Clin Invest. 1993 May;91:2155-68.

52. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. J Clin Invest. 1995 Sep;96:1395-403.

53. Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, Schmidt AM. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. J Clin Invest. 1996 Sep 1;98:1088-94.

54. Owen WF, Jr., Hou FF, Stuart RO, Kay J, Boyce J, Chertow GM, Schmidt AM. Beta 2-microglobulin modified with advanced glycation end products modulates collagen synthesis by human fibroblasts. Kidney Int. 1998 May;53:1365-73.

55. Sakaguchi T, Yan SF, Yan SD, Belov D, Rong LL, Sousa M, Andrassy M, Marso SP, Duda S, et al. Central role of RAGE-dependent neointimal expansion in arterial restenosis. J Clin Invest. 2003 Apr;111:959-72.

56. Che W, Asahi M, Takahashi M, Kaneto H, Okado A, Higashiyama S, Taniguchi N. Selective induction of heparin-binding epidermal growth factor-like growth factor by methylglyoxal and 3-deoxyglucosone in rat aortic smooth muscle cells. The involvement of reactive oxygen species formation and a possible implication for atherogenesis in diabetes. J Biol Chem. 1997 Jul 18;272:18453-9.

57. Makita Z, Yanagisawa K, Kuwajima S, Bucala R, Vlassara H, Koike T. The role of advanced glycosylation end-products in the pathogenesis of atherosclerosis. Nephrol Dial Transplant. 1996;11 Suppl 5:31-3.

58. Vlassara H. Advanced glycation end-products and atherosclerosis. Ann Med. 1996 Oct;28:419-26.

59. Ceriello A. Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. Diabetes Nutr Metab. 1999;12:42-6.

60. Boel E, Selmer J, Flodgaard HJ, Jensen T. Diabetic late complications: will aldose reductase inhibitors or inhibitors of advanced glycosylation endproduct formation hold promise? J Diabetes Complications. 1995 Apr-Jun;9:104-29.

61. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation. 2003 Jan 7;107:139-46.

62. Lai YL, Aoyama S, Nagai R, Miyoshi N, Ohshima H. Inhibition of L-arginine metabolizing enzymes by L-arginine-derived advanced glycation end products. J Clin Biochem Nutr. 2010 Mar;46:177-85.

63. Dei R, Takeda A, Niwa H, Li M, Nakagomi Y, Watanabe M, Inagaki T, Washimi Y, Yasuda Y, et al. Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease. Acta Neuropathol. 2002 Aug;104:113-22.

64. Jono T, Kimura T, Takamatsu J, Nagai R, Miyazaki K, Yuzuriha T, Kitamura T, Horiuchi S. Accumulation of imidazolone, pentosidine and N(epsilon)-(carboxymethyl)lysine in hippocampal CA4 pyramidal neurons of aged human brain. Pathol Int. 2002 Sep;52:563-71.

65. Bar KJ, Franke S, Wenda B, Muller S, Kientsch-Engel R, Stein G, Sauer H. Pentosidine and N(epsilon)-(carboxymethyl)-lysine in Alzheimer's disease and vascular dementia. Neurobiol Aging. 2003 Mar-Apr;24:333-8.

66. Girones X, Guimera A, Cruz-Sanchez CZ, Ortega A, Sasaki N, Makita Z, Lafuente JV, Kalaria R, Cruz-Sanchez FF. N epsilon-carboxymethyllysine in brain aging, diabetes mellitus, and Alzheimer's disease. Free Radic Biol Med. 2004 May 15;36:1241-7.

67. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, et al. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem. 1995 Nov;65:2146-56.

68. Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF, Kowall N. Oxidative damage in Alzheimer's. Nature. 1996 Jul 11;382:120-1.

69. Munch G, Thome J, Foley P, Schinzel R, Riederer P. Advanced glycation endproducts in ageing and Alzheimer's disease. Brain Res Brain Res Rev. 1997 Feb;23:134-43.

70. Ulusu NN, Sahilli M, Avci A, Canbolat O, Ozansoy G, Ari N, Bali M, Stefek M, Stolc S, et al. Pentose phosphate pathway, glutathione-dependent enzymes and antioxidant defense during oxidative stress in diabetic rodent brain and peripheral organs: effects of stobadine and vitamin E. Neurochem Res. 2003 Jun;28:815-23.

71. Fernandes AF, Zhou J, Zhang X, Bian Q, Sparrow J, Taylor A, Pereira P, Shang F. Oxidative inactivation of the proteasome in retinal pigment epithelial

cells. A potential link between oxidative stress and up-regulation of interleukin-8. J Biol Chem. 2008 Jul 25;283:20745-53.

72. Wu M, Bian Q, Liu Y, Fernandes AF, Taylor A, Pereira P, Shang F. Sustained oxidative stress inhibits NF-kappaB activation partially via inactivating the proteasome. Free Radic Biol Med. 2009 Jan 1;46:62-9.

73. Shang F, Taylor A. Ubiquitin-proteasome pathway and cellular responses to oxidative stress. Free Radic Biol Med. 2011 Apr 8.

74. Zhang X, Zhou J, Fernandes AF, Sparrow JR, Pereira P, Taylor A, Shang F. The proteasome: a target of oxidative damage in cultured human retina pigment epithelial cells. Invest Ophthalmol Vis Sci. 2008 Aug;49:3622-30.

75. Friedman DS, O'Colmain BJ, Munoz B, Tomany SC, McCarty C, de Jong PT, Nemesure B, Mitchell P, Kempen J, Group. EDPR. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol. 2004;122:564-72.

76. Frick KD, Gower EW, Kempen JH, Wolff JL. Economic impact of visual impairment and blindness in the United States. Arch Ophthalmol. 2007 Apr;125:544-50.

77. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002 Jun 15;359:2072-7.

78. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in

patients with impaired glucose tolerance: the STOP-NIDDM trial. Jama. 2003 Jul 23;290:486-94.

79. Ferland A, Brassard P, Lemieux S, Bergeron J, Bogaty P, Bertrand F, Simard S, Poirier P. Impact of high-fat /low-carbohydrate, high-, low-glycaemic index or low-caloric meals on glucose regulation during aerobic exercise in Type 2 diabetes. Diabet Med. 2009 Jun;26:589-95.

80. Jenkins DJ, Srichaikul K, Kendall CW, Sievenpiper JL, Abdulnour S, Mirrahimi A, Meneses C, Nishi S, He X, et al. The relation of low glycaemic index fruit consumption to glycaemic control and risk factors for coronary heart disease in type 2 diabetes. Diabetologia. 2011 Feb;54:271-9.

81. Kostolanska J, Jakus V, Barak L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2009 May;22:433-42.

82. DiGirolamo M. Cellular, metabolic, and clinical consequences of adipose mass enlargement in obesity. Nutrition. 1991 Jul-Aug;7:287-9.

83. Prevalence of Overweight, Obesity, and Extreme Obesity Among Adults: United States, Trends 1960–1962 Through 2007–2008 In: Statistics CNCfH, editor.; 2010.

84. Chiu CJ, Taylor A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. Prog Retin Eye Res. 2011 Jan;30:18-53.

85. Chiu C-J, Liu, S., Willett, W. C., Wolever, T.M.S., Brand-Miller, J. C. Barclay, A. C., A. Taylor. Informing food choices and health outcomes by use of the dietary glycemic index Nutrition Reviews. 2011 April;69:231-42.

86. Unoki-Kubota H, Yamagishi S, Takeuchi M, Bujo H, Saito Y. Pyridoxamine, an inhibitor of advanced glycation end product (AGE) formation ameliorates insulin resistance in obese, type 2 diabetic mice. Protein Pept Lett. 2010 Sep;17:1177-81.

Chapter 6. Appendix

In this exploratory pilot study, we evaluated a large number of parameters to begin to understand the relationship between dietary GI and AMD. To maintain a clear focus for each of the papers included in this thesis, all of the data was not presented in Chapters 3 and 4. The table below describes additional research questions that we addressed, with the corresponding results (**Appendix Table 1**). These research questions should be asked again, in a larger cohort of animals.

6.1 Table

Appendix Table 1. Additional results.

Research Question	Experimental Results
Does dietary GI modulate accumulation of C5b-9 in the retina?	No. We did not observe any difference in accumulation of C5b-9 in retinas of mice fed diets of different GI. However, other components of the complement pathway may be affected by AGE accumulation in the retina or other tissues. Investigation of the expression of other complement proteins, as well as other indicators of inflammation such as activated microglia, in the retinas of high and low GI-fed mice will help clarify whether GI-related risk for AMD is modulated by inflammation.
Does HQ modulate accumulation of AGEs or protein carbonyls in tissue?	17-month-old mice fed high GI diets with HQ accumulated more AGEs in the kidney, but less protein carbonyls in the heart, than age-matched mice fed high GI diets without HQ. Should these results be confirmed in a larger study, this variability in the effect of HQ on markers of oxidative stress suggests that the magnitude of stress and the particular stress response pathways activated by HQ may vary between organs.
Does dietary GI modulate levels of endogenous ubiquitin in tissue?	No. We did not find any GI-related difference in levels of ubiquitin conjugates in any of the tissues analyzed. Dietary GI may still be modulating UPS activity, but changes in one end of the ubiquitin enzymatic cascade (such as proteasome activity) may be compensated by changes at the other end of the pathway (such as ubiquitin conjugate formation). Further investigation of each of these activities would clarify the particular stress of dietary GI on the UPS.
Does dietary GI modulate levels of C-Reactive Protein in plasma?	No. The relationship between CRP and dietary GI in mice has not been explored previously, and so this analysis should be repeated in a larger cohort of high and low GI-fed mice to confirm our preliminary findings.