Eye Disease, Nutrition and the Environment

Nutrition and the Environment in Eye Disease

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Rancho Valencia, California

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Preface

The fifth in a series of symposia on accelerating the implementation of research results on eye disease was held on June 24–25, 2007, at Rancho Valencia, California. The theme of this Fifth Drabkin Eye Disease Symposium was recent research results on the role of nutrition and other environmental factors in the chronic eye diseases that are major causes of blindness in the United States and around the world. The 11 symposium participants (listed in appendix A) were selected to provide expertise on research progress and prospects for translation to practice for diseases of the cornea and lens, diabetic retinopathy, age-related macular degeneration, and retinitis pigmentosa. Two of the participants provided a focus on the emerging importance of lipids, particularly docosahexaenoic acid and its derivatives, in eye health and potentially for slowing progression in cases of chronic blinding disease. Because dietary supplements as complementary or alternative medicines are so closely interwoven with patient interests in nutritional factors in eye disease, two participants focused their presentations on the scientific evidence concerning alternative therapies.

The participants' presentations on their assigned topics, together with extensive discussion of the presentations, occupied the first day of the symposium. On the second day, the participants proposed conclusions and recommendations for consideration by the entire group. The group's discussions provided the basis for the 16 conclusions and recommendations listed in table 1. Section 1 provides supporting explanation for the conclusions and recommendations. Section 2 summarizes the presentations by the participants on specific topics (see appendix B for

the symposium agenda), highlighting the research evidence supporting the conclusions and recommendations.

Like the prior symposia on glaucoma, age-related macular degeneration, emerging therapies for diseases of the retina and optic nerve, and eye diseases in diverse populations [1–4], this symposium was made possible by the endeavors of Robert Drabkin of Los Angeles, and supported by the UCLA Support Group of the Jules Stein Eye Institute. The symposium and report preparation were organized and overseen by the Washington Advisory Group. This report was prepared with the guidance and supervision of the symposium co-chairs, who are responsible for its technical content.

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Acronyms

AGE	advanced glycation end-product
AIF	apoptosis inducing factor
AMD	age-related macular degeneration
AMP	adenosine 5'-monophosphate
ARA	arachidonic acid
AREDS	Age-Related Eye Disease Study
CAM	complementary and alternative medicine
CFH	complement factor H
COAG	chronic open-angle glaucoma
CREB	cyclic-AMP-response-element-binding protein
DHA	docosahexaenoic acid
DR	diabetic retinopathy
FDA	U.S. Food and Drug Administration
IOP	intraocular pressure
LOCS	Lens Opacities Classification System III
MnTBAP	manganese(III) 5,10,15,20-tetrakis(4-benzoic acid)porphyrin
MPTP	mitochondrial permeability transition pore
NCCAM	National Center for Complementary and Alternative Medicine
NEI	National Eye Institute
NEHEP	National Eye Health Education Program
NO•	nitric oxide radicals
NOS	nitric-oxide-synthase
NPD1	neuroprotectin D1 [10R, 17S docosatriene, a dyhydroxlated derivative of DHA]
PAR	population-attributable risk
PARP	poly-ADP ribose polymerase
PDR	proliferative diabetic retinopathy

PEDF	pigment epithelium–derived factor
PUFA	polyunsaturated fatty acid
RDA	recommended daily allowance
RP	retinitis pigmentosa
RPE	retinal pigment epithelium
USP	United States Pharmocopoeia
UV	ultraviolet
VEGF	vascular endothelial growth factor
VEP	visual evoked potential

Executive Summary

The Fifth Drabkin Symposium focused on current and potential opportunities to fight blinding eye disease by means of improved nutrition and attention to environmental risk factors. The proportion of Americans affected by major organic causes of blindness and low vision is projected to increase significantly as the population ages. If past trends in disease progression can be slowed down through a combination of prevention and more-effective treatment, we can achieve substantial benefits in terms of both the quality of life for the individuals affected and reduced public health expenditures to deal with the societal consequences of blindness. With variations dependent on level of economic development, ethnocultural differences, and other factors, *every developing and developed nation* faces this challenge of increased burdens from chronic eye disease.

Modifying nutrient intake and other environmental factors offers the potential for highly cost-effective approaches to meeting the challenge of vision loss from chronic eye disease. In particular, interventions to change diet and environmental risk factors early in the disease process, rather than waiting to remediate disease consequences at a later stage, is not only cost-effective but will also enhance the quality of life for patients and their families.

Nutrition, Environment, and Corneal Disease

Around the world, diets deficient in vitamin A continue to be a leading cause of corneal disease and preventable blindness. Proven interventions

exist to provide the small amount of vitamin A supplementation needed just twice a year to prevent this threat to vision. The participants in the Fifth Drabkin Symposium agreed that increased support for effective programs already in operation can help in meeting this challenge. For Americans and other populations in which vitamin A deficiency is rare, modifying dietary intake of polyunsaturated fatty acids (PUFAs) may play a role in treating dry eye syndrome. In the United States and other countries where surgery to correct refractive error has become popular, the long-term health implications of this procedure remain inadequately studied, even as millions choose to undergo this elective procedure.

The cornea, which is exposed to the environment on the surface of the eye, absorbs most of the ultraviolet (UV) light in sunlight. Many studies over the years have documented the damaging effects on the cornea of UV-B light in particular. Starting from childhood, the simple act of protecting the eyes with sunglasses can significantly decrease the risk of corneal damage.

Nutrition, Environment, and Cataract

Buildup of damaged proteins and other toxic constituents within the crystalline lens of the eye causes loss of transparency and formation of cataracts. Biomolecules are being identified, many of which are antioxidants, that prolong and enhance the normal mechanisms for removing these damaged proteins as they form, thus avoiding or slowing down the buildup that leads to cataract. Dietary supplementation of antioxidants to intervene in the biochemical pathways involved in eye disease progression was a recurring theme at the Fifth Drabkin Symposium, and the possibility of slowing cataract formation by increasing the local concentrations of these bioactive molecules was an intriguing example of how an appropriate dietary supplement may improve eye health. On the negative side, higher levels of simple sugars and other simple carbohydrates appear to increase the rate of damage to the bulk proteins essential to normal lens functioning and to compromise the mechanisms for removing damaged proteins. Thus, decreasing the dietary intake of these nutrients may lower the risk of cataract, as well as of other ocular and systemic diseases.

Exposure to sunlight, particularly in the UV-B region, is a major environmental risk factor for cataract, as well as for corneal disease. The risk is highest for those with outdoor occupations that require exposure to strong sunlight, including the reflected glare from water or snow. Proper sunglasses, or even wearing a brimmed hat, can substantially reduce the risk of cataract, which each year causes about 1.5 million new cases of blindness worldwide.

Nutrition, Environment, and Diabetic Retinopathy

Diabetic retinopathy is a rising cause of blindness in America for two reasons. First, more Americans have lifestyles that increase their risk of developing diabetes. Second, even after they develop type 2 diabetes, many people fail to control their blood sugar level adequately. Strict control of blood sugar is essential to preventing the damage to the capillaries of the retina that leads to the progressively more severe stages of diabetic retinopathy. The best hope for stemming the rising tide of diabetes-related blindness and vision loss is motivating diabetes patients to control their blood sugar and providing them with more-effective means to do so. Diet control, regular exercise, and periodic screening of diabetic individuals for initial signs of retinal damage are essential parts of a comprehensive public health strategy to deal with America's diabetes epidemic.

Nutrition, Environment, and Age-Related Macular Degeneration

An increasing body of biomedical evidence supports the view of age-related macular degeneration (AMD) occurrence as largely a matter of genetically determined risk factors. Nevertheless, modifying environmental risk factors, which interact with these genetic susceptibilities, does appear to alter the risk of disease progression to advanced AMD, in which severe vision loss or blindness are common outcomes. Scientific studies uniformly agree that smoking tobacco is a significant environmental risk for AMD. Pursuing a healthy diet and not smoking continue

to be essential recommendations for those at increased risk of AMD because of genetic susceptibility. Despite disparities among major studies on the environmental risk factors—most of which seem to reflect differences in study design—consumption of foods rich in omega-3 PUFAs, such as fish, and higher levels of dietary intake of zinc and certain antioxidants, such as vitamin E, appear to lessen the risk of AMD progression. The Age-Related Eye Disease Study of the National Eye Institute has shown that combinations of zinc and antioxidants can slow AMD progression.

Antioxidants and Retinitis Pigmentosa

The role of nutrition as a contributing factor in obviously genetic diseases such as retinitis pigmentosa (RP) is just now emerging. Recent laboratory studies indicate that antioxidants are indeed effective in slowing retinal degeneration in animal models for RP and that combinations of antioxidants in a dietary supplement may be more effective than any one antioxidant alone. If clinical trials show that a nutritional supplement can slow the progression of photoreceptor loss in RP or similar diseases of the retina, then studies of nutritional habits and potential nutritional deficits, by geographic region and ethnocultural group, will be valuable for determining which groups at risk for RP (or other retinal diseases) are likely to benefit from taking a supplement, as well as which at-risk groups are unlikely to benefit.

Emerging Roles for Omega-3 Fatty Acids in Eye Health

The omega-3 PUFAs, particularly docosahexaenoic acid (DHA) and its bioactive derivatives, are necessary to the developing retina and brain and have essential roles in preserving normal functioning of the retina's photoreceptor cells. Ensuring that pregnant and nursing mothers, as well as bottle-fed infants, receive an adequate dietary intake of DHA will enhance maturation of retinal function, visual acuity, and overall neurological performance. The DHA derivative neuroprotectin D1, which is synthesized within the cells of the retinal pigment epithelium (RPE),

appears to protect both photoreceptor and RPE cells from oxidative stress and from neurodegeneration resulting from oxidative damage.

Prospects for an Intervention Strategy for Multiple Degenerative Diseases of the Retina

During their discussions of several presentations on RP, AMD, and photoreceptor cell functioning in the retina, the participants focused on the growing evidence that specific bioactive molecules—including antioxidants and a derivative of DHA—appear to interfere in the pathways that cause photoreceptor cells to die off in response to stressing conditions. There seem to be commonalities across the disease pathways of AMD, RP, glaucoma, and perhaps even diabetic retinopathy that could be exploited by a general intervention strategy aimed at slowing or preventing disease progression by interfering with this process of programmed cell death, or *apoptosis*. The participants agreed on the importance of a systematic exploration of the use of combinations of antioxidants as a dietary intervention in disease progression by retinal cell apoptosis.

Nonconventional Therapies and Eye Health

Americans want to find dietary and environmental solutions to their vision-related ailments and the perceived risks of failing vision. They are seeking solutions in complementary and alternative medicine in increasing numbers and with increasing per capita expenditures. Special diets or dietary supplements are often recommended by nonphysicians or marketed by commercial entities as potentially therapeutic for one or more of the major forms of chronic eye disease. Little or no scientific evidence supports many of these claims. Given the potential value of nutritional approaches to ameliorating chronic disease of many kinds and the propensity of the lay population to seek solutions beyond conventional medicine, diligence in evaluating claims of efficacy is the responsibility of patients, their physicians, and regulatory agencies. Investigations of nonconventional therapies, whether as alternative or complementary treat-

ments, must adhere to the scientific principles of evidence-based medical practice. For example, in the case of complementary and alternative therapies for glaucoma, the only option that has scientific support is to *complement* the glaucoma patient's conventional medical treatment with routine, healthy exercise.

Table 1. Principal Conclusions and Recommendations of the Fifth Drabkin Symposium

Overarching Themes
1. Vision loss and blindness due to diseases in the anterior or posterior segments of the eye such as cataract, glaucoma, diabetic retinopathy, age-related macular degeneration, corneal disease, and retinitis pigmentosa result in costly burdens on both a public health and personal basis. Virtually all the elderly are affected by some form of vision-threatening condition. The proportion of those affected is projected to grow rapidly, especially in developed countries, as the proportion of elderly and their life expectancy increase.
2. Additional studies to clarify how patterns of nutrient intake and environmental influences affect chronic eye diseases can have a substantial effect on improving not only ocular health but also the health of many other organ systems. The benefits in prolonging visual function by even a few years for the elderly would substantially diminish public health care costs. Addressing the roles of nutrient intake and environment, including socioeconomic factors, early in the disease process, rather than trying to remediate in a later stage of disease, is not only cost-effective but will also enhance the quality of life for our elderly.
3. As explored in depth at the Fourth Drabkin Symposium in 2005, eye diseases of complex origin (multifactorial eye disease) often present differently in population groups that differ in ethnic, socioeconomic, or other measurable parameters. A growing body of evidence indicates that differences in genetic disposition contribute to these differences among groups. Similarly, environmental factors, including cultural differences such as diet preferences, appear to contribute to these differences among population groups.
4. Closely associated with questions about the role of nutrition and environment in eye disease is the broad area of nonconventional therapies used either as an alternative to conventional therapy or as an additional treatment (complementary therapy). In particular, special diets or dietary supplements are often recommended by nonphysicians or marketed by commercial entities as potentially therapeutic for the major forms of chronic eye disease. The symposium participants agreed on the following points for evaluating claims made for nonconventional therapies:

	(a) Investigations of nonconventional therapies, whether as alternative or complementary treatment, must adhere to the fundamentals of sound scientific research, including hypothesis-driven questions and methods based on appropriate approaches. In short, scientific proof of principle must be established. (b) Thus far, most of the claims of therapeutic efficacy for alternative therapies have not been substantiated by rigorous scientific tests.
	Nutrition, Environment, and Corneal Disease
5.	Severe damage to the cornea from nutritional deficiency (e.g., xerophthalmia from inadequate vitamin A) or environmental factors (e.g., ultraviolet exposure) is well established. Proven interventions to prevent such damage exist and deserve expanded support.
6.	Other issues of nutrition (e.g., treatments for dry eye using polyunsaturated fatty acids or other bioactive lipids) and environmental conditions (e.g., long-term effects of refractive surgery) require further careful study to establish the evidence base adequate to support suggested new eye health practices.
	Nutrition, Environment, and Cataract
7.	Damaged proteins and other toxic constituents of the lens are normally removed by the protein-editing machinery within the cells. These damaged residues are deleterious to normal lens function if they accumulate, leading to loss of transparency and cataract formation. Recent research has identified biomolecules, many of which are antioxidants, that are active in preserving lens function by helping to remove or detoxify these residues, thus delaying damage to the bulk proteins essential to normal lens function.
8.	Even nutrients normally thought of as "safe" can have deleterious effects. Simple sugars, for example, can damage the bulk proteins of the lens and compromise cellular editing. Maintaining adequate levels of omega-3 fatty acids appears to aid in the health of the lens as well as other ocular tissues. Thus, a prudent preventive strategy for cataract includes optimizing diet to prolong healthy cellular function. To support this preventive strategy, further work is needed to examine systematically the potential of other common nutrients for adverse or beneficial effects on lens transparency.

Nutrition, Environment, and Diabetic Retinopathy
9. Diabetic retinopathy (DR) is an important and rising cause of blindness in the United States and around the world. Systemic management of diabetes and prevention of retinopathy are perhaps the most effective approaches for reducing diabetic blindness in the future.
10. Knowledge of the risk factors for DR and diabetes in general is important in counseling patients and in devising therapeutic approaches. (a) Diet control is a critical factor not only in managing the disease and its progressive vision loss consequences but often in preventing diabetes from developing. (b) Early screening at appropriate intervals is key to the management of DR. (c) The sociocultural environment is clearly important in development of type 2 diabetes and of DR in all forms of the disease. These sociocultural factors have not yet been sufficiently investigated. Further research is needed into how these factors translate into barriers to ideal prevention and management of DR for the general population and for special populations.
Nutrition, Environment, and AMD
11. AMD is a multifactorial disease in that both gene mutations and environmental factors contribute to the disease process. Data on gene-environment interactions imply that the increased risk of AMD associated with some alleles may be ameliorated by healthy lifestyles. In general, since environmental factors are modifiable, maintaining a healthy diet and not smoking are still essential recommendations for those at increased risk.
12. Due to differences in many published studies in design and outcome, the body of data on the effects of diet on AMD risk appears largely inconsistent at present. As in the case of cataract, additional rigorous studies are needed to determine the efficacy and safety of other dietary modifications.
13. The AREDS results show that combinations of antioxidants can be effective in slowing AMD progression. These antioxidants may interfere with the pathways of programmed cell death (apoptosis) in the retina, which are associated with loss of photoreceptor cells in retinitis pigmentosa as well as AMD. A general intervention for these pathways of programmed cell death and photoreceptor loss should be useful in preventing or slowing the progression of a range of diseases besides AMD—including retinitis pigmentosa, glaucoma, and even diabetic retinopathy.

Nutrition, Environment, and Retinitis Pigmentosa

14. A small positive effect of vitamin A in slowing vision loss for some retinitis pigmentosa (RP) patients has been known for some years. However, the finding that combinations of antioxidants slow vision loss from RP in animal models is an exciting new development and should be explored systematically in both preclinical studies and clinical trials. If a nutritional supplement formulation is found to be beneficial in slowing the progression of photoreceptor loss in RP or other diseases of the retina, a study of the nutritional habits and potential nutritional deficits of populations in different geographical areas and of different cultural backgrounds will be important to undertake. Such a study would be valuable for addressing the following questions:

    (a) Which special populations are likely to benefit from taking a nutritional supplement?

    (b) Which supplement formulation is likely to be needed over an entire lifetime?

    (c) Which special populations might not benefit?

Crosscutting Issues

15. **The Emerging Importance of Lipids and Their Bioactive Products.** Evidence from the cellular and molecular biology of the normally functioning eye, together with evidence from the pathophysiology of some retinal diseases and from animal models of retinal degeneration, supports an important role for long-chain polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA), in photoreceptor physiology.

    (a) Biologically active molecules derived from DHA, called docosanoids, appear to play significant roles in protecting retinal cells, including the photoreceptors, from the damaging effects of inflammation and programmed cell death.

    (b) DHA is a critical building block from before birth through early childhood. With more recent data on the ratio in breast milk of DHA (an omega-3 fatty acid) to omega-6 fatty acids, the ratio of DHA to arachidonic acid in commercial infant formulas should be increased and new products that provide a dietary source of DHA in the second year of life should be developed.

16. **Nonconventional Therapies: What Works and What Doesn't.** Substantial numbers of patients with chronic eye disease use nonconventional treatments to complement, or as an alternative to, treatment prescribed by a physician. The scale of such use is great enough to raise significant issues of safety and efficacy—including the cost of expenditures on these treatments. In light of the substantial use of nonconventional treatments by Americans suffering from or concerned about eye disease, the symposium participants supported the following conclusions:

    (a) Although many factors are likely to contribute to this substantial interest in and consumer expenditure on nonconventional treatments, there appears to be no systematic study of why people turn to them.

    (b) Given this societal context, health care providers and educators should learn if a patient is using a nonconventional therapy and be informed about the scientific evidence (or lack thereof) for both efficacy and potential harm associated with its use as an alternative or complement to conventional treatment. Potential harm includes any direct negative (e.g., toxic) effects of the agent or procedure and the economic cost of ineffectual treatments, as well as the consequences if the patient ceases to follow a conventional therapeutic regimen. The symposium participants suggested that a provider's responsibility begins but does not end with informing the patient about any safety and efficacy issues related to a nonconventional therapy. Unfortunately, health care providers or marketers often fail to carry through on this responsibility.

    (c) As an example, in the treatment of glaucoma, no scientifically sound evidence exists to support the claims made for a variety of nonconventional therapies, including the use of vitamins, herbs such as gingko biloba and bilberry (purported to be beneficial antioxidants), acupuncture, meditation, or special diets. In distinct contrast to these therapies that have inconclusive or no supporting evidence for efficacy, both short-term and moderate long-term exercise have been shown to reduce intraocular pressure. Thus, exercise should be considered as useful complementary therapy to accompany the standard medical treatments for glaucoma.

1. Conclusions and Recommendations

This section discusses the principal conclusions and recommendations, collected in table 1, that resulted from participant discussions at the Fifth Drabkin Symposium.

Following the organization used in table 1, four overarching themes are discussed first. The next five subsections focus on the conclusions and recommendations related to nutritional and environmental factors in particular eye diseases: corneal disease, cataract, diabetic retinopathy (DR), age-related macular degeneration (AMD), or retinitis pigmentosa (RP). Two topics presented and discussed at the symposium are relevant to several of these major diseases: the emerging importance of dietary lipids and their bioactive products, and the evidence for and against the efficacy and safety of a range of nonconventional therapies. The final subsection expands on the symposium conclusions and recommendations concerning these crosscutting topics.

Overarching Themes

Four overarching themes on the role of nutrition and environmental factors emerged from the presentations and discussions during the symposium.

1. **Vision loss and blindness due to diseases in the anterior or posterior segments of the eye such as cataract, glaucoma, diabetic retinopathy, age-related macular degeneration, corneal disease, and retinitis pigmentosa result in costly burdens on both a public health and personal basis. Virtually all the elderly are affected**

by some form of vision-threatening condition. The proportion of those affected is projected to grow rapidly, especially in developed countries, as the proportion of elderly and their life expectancy increase.

Serious vision impairment or blindness is a threat we intuitively fear. In a recent worldwide study of attitudes toward glaucoma and blindness, twice as many people were afraid of going blind as were afraid of dying prematurely [5]. The personal costs of vision impairment including blindness are borne by an estimated 3.4 million Americans age 40 and older—2.85 percent of that age group. A million of these adults are blind [6, pp. 6–8; 10]. While diminished quality of life may be the principal driver of our fears of vision loss, the measurable economic costs to individuals are substantial as well. In 2003, the National Eye Institute (NEI) estimated that the direct costs to individuals of vision impairment were $48.7 billion per year, with another $18.9 billion in indirect costs to individuals (primarily in lost work time) [7]. In addition to this total cost to individuals of $67.6 billion, the annual cost to the federal government, in Medicare benefits paid and lost taxable income, of vision impairment and blindness is estimated at more than $4 billion [6, p. 4].

The principal chronic diseases of the eye tend to increase in prevalence and severity with age. This generality applies to the diseases on which this report focuses: corneal disease and cataract, AMD, DR, glaucoma, and RP. The following statistics illustrate the scale of the public health burden they cause, as well as the personal burden for afflicted individual Americans:

- **Cataract.** Each year, there are 400,000 new cases of cataract and a million cataract surgeries are performed on Americans. An estimated 20.5 million Americans age 40 and older—one person in six—are affected by this blinding disease. The federal government spends more than $3.4 billion each year for cataract treatment under Medicare. By age 80, more than half of all Americans have cataract [6, pp. 22–23].
- **AMD.** Thirteen million Americans have signs of macular degeneration, and 1.65 million Americans age 50 and older have late-stage AMD. Late-stage AMD includes both dry AMD (whose

end stage is geographic atrophy) and wet AMD (in which numerous new blood vessels form in the diseased area—neovascularization—and are prone to leakage and hemorrhage) [6, pp. 18–19]. More recently, the availability of effective antineovascular agents has substantially increased the number of therapeutic interventions.

- **Diabetic retinopathy.** More than 10 million Americans have been diagnosed with diabetes, and an estimated 5.4 million cases remain undiagnosed. DR—the blockage, breakdown, and leakage of the small blood vessels in the retina—is a common complication of diabetes. The longer an individual has diabetes, the more likely he or she is to begin developing DR. An estimated 5.3 million Americans suffer from this eye disease, which usually starts with an early stage called mild retinopathy. The progression to worse stages (such as definite nonproliferative retinopathy, macular edema, and proliferative retinopathy) can be slowed but not reversed or halted completely, even with strict control of blood sugar levels [6, pp. 26–27].
- **Glaucoma.** More than 2.2 million Americans age 40 and older have glaucoma, or about 1.9 percent of this population group. However, special populations are known to have seriously higher prevalences of glaucoma. For example, the prevalence in African American women is 4.8 percent, compared with 1.6 percent for American women of European descent [6, pp. 30–33].
- **Retinitis pigmentosa.** More than 100,000 Americans are thought to suffer from RP, and the estimate of worldwide prevalence is 1 person in every 3,400. Although RP is relatively rare, it often strikes at birth or early childhood, leaving an otherwise healthy individual with little or no vision for life.

For an individual with early signs of any of these chronic eye diseases, the risk of serious impairment increases as that person ages. As the proportion of elderly in America increases, the numbers of blind or visually impaired Americans will increase inexorably—unless we can find ways to slow or halt the progression of these diseases from initial susceptibility or first indications to eventual vision loss.

2. Additional studies to clarify how patterns of nutrient intake and environmental influences affect chronic eye diseases can have a substantial effect on improving not only ocular health but also the health of many other organ systems. The benefits in prolonging visual function by even a few years for the elderly would substantially diminish public health care costs. Addressing the roles of nutrient intake and environment, including socioeconomic factors, early in the disease process, rather than trying to remediate in a later stage of disease, is not only cost-effective but will also enhance the quality of life for our elderly.

The theme of the Fifth Drabkin Symposium was to explore the scientific evidence for whether what we eat (nutrient intake) and other environmental influences affect the course of these chronic eye diseases. Are there diets or dietary supplements that decrease the risk of vision loss from one or more of these ailments? Are there dietary patterns that increase the risk? And what is the scientific basis for the claims that are made for special diets, supplements, or other nonconventional treatments in preventing or ameliorating these chronic diseases?

After listening to the presentations summarized in section 2 of this report and discussing what the evidence from biomedical science means for these questions about nutrition and environmental influences in eye disease, the symposium participants concluded that additional studies were urgently needed to address what could become a "blindness epidemic" as our population ages. For example, basic work to elucidate the etiologies of these diseases could help define optimal levels or patterns of nutrient intake for those at risk. Although that knowledge is still tentative in many respects, the sense of the group was that some of the promising recent results will produce substantial insights into the use of nutrition to improve ocular health, as well as general body health. Furthermore, nutrition can play an important role in diminishing health care costs by prolonging visual function. Addressing nutritional deficits or potential interventions early in the disease process, rather than trying to remedy consequences at a later stage of disease development, has the potential to provide care that is much more cost-effective from both personal and public health perspectives. Early attention to nutritional factors can enhance the quality of life—vision, mobility, and indepen-

dence—for many of the elderly. Exploiting nutrition to maintain and prolong lens and retina function requires no new technology and is currently possible with only minor behavioral change.

Examples of this general principle about the importance of attention to nutritional factors *early in life and throughout it* are presented in each of the subsections below on specific eye diseases, as well as in the summaries in section 2 of the symposium presentations. One such example is the accumulating evidence that diets high in simple carbohydrates, such as the cane sugar (sucrose) or high-fructose sweeteners added to beverages and many packaged foods, increase the concentrations of metabolic products that damage cell proteins and compromise normal cellular functions, including repair processes. These effects are being observed in ocular tissues, as well as systemically (as in diabetes mellitus) in other tissues and organs. A diet optimized to limit the amount of simple carbohydrates can prolong normal cellular functions in the eye and, indeed, throughout the body. Such a diet is therefore an essential part of a prudent strategy for preventive medicine. Conversely, a diet high in antioxidants may slow or even halt the progression of pathology seen in AMD and could add years of useful vision to the lives of patients with inherited retinal degenerations such as RP.

3. As explored in depth at the Fourth Drabkin Symposium in 2005, eye diseases of complex origin (multifactorial eye disease) often present differently in population groups that differ in ethnic, socioeconomic, or other measurable parameters. A growing body of evidence indicates that differences in genetic disposition contribute to these differences among groups. Similarly, environmental factors, including cultural differences such as diet preferences, appear to contribute to these differences among population groups.

The Fourth Drabkin Symposium report emphasized that the ethnic categories typically used in demography, as well as in everyday discourse (e.g., Black, White, Latino, Asian), are, from a scientific perspective, *at best* markers for underlying factors that could account for the observed differences in how a disease occurs within special groups within a general population [4, p. 50]. These underlying factors can include not only

genetic differences—which were of particular interest in the Fourth Drabkin Symposium—but also cultural (lifestyle) and environmental differences. Among the latter are the nutrition factors that are the focus of this report.

The rapidly developing field of *genetic epidemiology* emphasizes the fundamental truth that genetic differences among individuals often find expression in differing disease susceptibilities in response to environmental factors, including diet and lifestyle differences. Conversely, both the beneficial and the adverse effects of environmental factors, including but not limited to the effects of diet and nutrition, may apply to some individuals more than others because of genetic differences in response to those factors. To make the most of nutrition as an active component of a total health care approach that includes eye health throughout life, we need to decipher these complex interactions between "what's in our genes" and what we expose ourselves to during life. The previous Drabkin Symposium emphasized that the differences in disease experience among special populations can be a valuable tool for discerning and testing the patterns of genetic susceptibility, environmental influences, and differential risk of disease. The latest Drabkin Symposium follows up on that theme, asking what is definitively known—and what is not scientifically supported though sometimes claimed—about the specific effects of nutrition and the environment on the general population and on special groups.

4. Closely associated with questions about the role of nutrition and environment in eye disease is the broad area of nonconventional therapies used either as an alternative to conventional therapy or as an additional treatment (complementary therapy). In particular, special diets or dietary supplements are often recommended by nonphysicians or marketed by commercial entities as potentially therapeutic for the major forms of chronic eye disease. The symposium participants agreed on the following points for evaluating claims made for nonconventional therapies:

(a) Investigations of nonconventional therapies, whether as alternative or complementary treatment, must adhere to the fundamentals of sound scientific research, including hypothesis-driven questions and methods

based on appropriate approaches. In short, scientific proof of principle must be established.

(b) Thus far, most of the claims of therapeutic efficacy for alternative therapies have not been substantiated by rigorous scientific tests.

The National Center for Complementary and Alternative Medicine (NCCAM, a center within the National Institutes of Health) defines *complementary and alternative medicine* (CAM) as

> a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Conventional medicine is medicine as practiced by holders of M.D. (medical doctor) or D.O. (doctor of osteopathy) degrees and by their allied health professionals, such as physical therapists, psychologists, and registered nurses [9].

- *Complementary medicine* is used *together with* conventional medicine. An example of a complementary therapy is using aromatherapy to help lessen a patient's discomfort following surgery.
- *Alternative medicine* is used *in place of* conventional medicine. An example of an alternative therapy is using a special diet to treat cancer instead of undergoing surgery, radiation, or chemotherapy that has been recommended by a conventional doctor.

In agreement with these NCCAM definitions, this report uses the term "alternative therapy" to refer to any therapeutic approach that is not presently considered to be part of conventional medicine and that is used in place of conventional treatment for one of the eye diseases covered in the report. If a nonconventional therapy is used to supplement, rather than replace, conventional treatment, it is being used as complementary therapy. In either case, the key question is "What is the evidence for the efficacy (and sometimes safety) of the nonconventional therapy, whether used as an alternative to or to complement conventional treatment?"

Note that a therapy's status at present as an alternative or complementary therapy does not preclude it becoming accepted as a conventional therapy if adequate scientific evidence can be accumulated to

support the claims made for its therapeutic effects. Indeed, the primary interest of this report in alternative therapies is to examine the current status of efforts to support their claims of therapeutic value and to encourage proper testing for any such therapy that shows promise. The NCCAM states the issue of the scientific basis for CAM claims as follows:

> Some health care providers practice both CAM and conventional medicine. While some scientific evidence exists regarding some CAM therapies, for most there are key questions that are yet to be answered through well-designed scientific studies—questions such as whether these therapies are safe and whether they work for the diseases or medical conditions for which they are used [9].

Nutrition, Environment, and Corneal Disease

5. Severe damage to the cornea from nutritional deficiency (e.g., xerophthalmia from inadequate vitamin A) or environmental factors (e.g., ultraviolet exposure) is well established. Proven interventions to prevent such damage exist and deserve expanded support.

Based on the presentation by Dr. Sheila West on the ongoing battle against the effects of vitamin A deficiency in developing countries, the symposium participants agreed on the importance of continuing U.S. governmental and private support for the international programs at the forefront of this worldwide effort (figure 1). UNICEF and several nongovernmental organizations have been engaged for a number of years in programs for vitamin A supplementation and food fortification aimed at reducing xerophthalmia, keratomalacia, and child mortality. Xerophthalmia, which results mainly from a severe systemic deficiency of vitamin A, is a condition in which the outside of the eye becomes dry, thickened, and lusterless. This early-stage of vitamin A deficiency affects the conjunctiva and cornea and can be reversed with immediate vitamin A supplementation in the diet. Untreated, it is often the first warning sign of imminent irreversible damage and loss of vision in the individual child.

Figure 1. Prevalence and Severity of Xeropththalmia (x) and Vitamin A Deficiency (VAD)

Source: Dr. Sheila West, presentation to the Fifth Drabkin Symposium, June 2007, from World Health Organization data.

Xerophthalmia in children is also a marker for vitamin A deficiency in the community. Keratomalacia, in which the cornea is softened and ulcerated, also results from severe systemic deficiency of vitamin A. This stage of cornea dystrophy often leads to wholesale extrusion of the contents of the eyeball through the ulcerated cornea and thus causes total and irreversible blindness.

Because the body retains vitamin A for a considerable time, storing it in the liver and recovering its bioactive forms for reuse, a single vitamin A pill given twice a year is enough to prevent deficiency in children and adults. The UNICEF-sponsored programs emphasize providing treatment to nursing women as well as to children, as an infant's supply of vitamin A can be maintained through breastfeeding. The challenge for these programs is in delivering the semiannual dosage to those who need it, as the delivery cost is typically 10 times greater than the cost of the vitamin A supplement pill. With respect to U.S. governmental support for international programs that distribute vitamin A, the United States Agency for International Development has been the principal funding mechanism. The symposium participants agreed that this federal agency should be encouraged to do more to support vitamin A supplementation programs worldwide.

Exposure of the cornea to the ultraviolet (UV) portion of normal sunlight is an environmental factor that is known to damage the cornea as well as the lens (see the discussion below of cataract and UV exposure). The symposium participants recommended that the National Eye Health Education Program (NEHEP), in which the NEI participates with a number of optometric and ophthalmologic organizations as partners, could be used more effectively to promote protection of the cornea and lens from UV exposure. The NEHEP partners could, for example, expand their collaboration with existing "sun-safe" campaigns for limiting skin exposure to UV radiation, such as the campaign supported by the U.S. Environmental Protection Agency. The sun-safe campaigns in Canada, Australia, and the United States currently emphasize dermatologic consequences such as the skin cancers melanoma and basal cell carcinoma. NEHEP involvement could encourage these programs to include appropriate emphasis on risks to the cornea and lens.

6. Other issues of nutrition (e.g., treatments for dry eye using polyunsaturated fatty acids or other bioactive lipids) and environmental conditions (e.g., long-term effects of refractive surgery) require further careful study to establish the evidence base adequate to support suggested new eye health practices.

Beyond the well-known role of vitamin A deficiency as the cause of xerophthalmia and parallel ocular and systemic disease, other potential linkages between nutrition and corneal disease are suggestive but not yet firmly established. For example, the symposium participants agreed that research needs to continue into the role of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) and other bioactive lipids in dry eye. Dry eye is a condition associated with inadequate tear production and marked by redness of the conjunctiva and by itching and burning sensations of the eye. Dr. West's presentation on this condition emphasized that its linkage with insufficient presence in the cornea of specific biolipids may be limited to particular forms, or phenotypes, of dry eye. Establishing a definitive link will require careful epidemiologic differentiation of these phenotypes, rather than lumping all forms of dry eye together. For dry eye phenotypes in which a connection can be established, a nutritional approach, including dietary supplementation, may prove an effective treatment.

One obstacle to an effective differentiation of dry eye phenotypes is the variability in how dry eye is characterized in epidemiologic studies. The symposium participants agreed that standardization across survey instruments is important to making progress in identifying distinct phenotypes of dry eye and establishing which forms may be mitigated by supplementing the diet with specific PUFAs.

Dry eye occurs more frequently in women than men. An unresolved question is whether estrogen loss—for example, in menopause—is a factor in this gender difference. If this potential association is confirmed by further study, then dietary phytoestrogens, such as those found in some soy products, could prove useful in treating specific dry eye phenotypes. The symposium participants concluded that exploration of this issue is warranted, but basic research to determine proof of principle is needed. With respect to the symposium's concern with complementary

and alternative therapies; see conclusions 4 and 16), this possible role for nutritional phytoestrogens in alleviating some forms of dry eye is already being touted in the marketplace, despite the lack of confirming scientific evidence.

Another potential role for PUFAs and other bioactive lipids is in wound healing and long-term corneal health following refractive surgery. The participants expressed concern with the widespread and rapidly growing use of refractive surgery as an alternative to conventional corrective lens prescriptions to enhance visual acuity. The long-term consequences of this technique have not been thoroughly tested in clinical studies. They recommended that a baseline cohort of refractive surgery patients be established for a long-term study—one that follows the study subjects into older age—to assess adverse outcomes.

Nutrition, Environment, and Cataract

7. Damaged proteins and other toxic constituents of the lens are normally removed by the protein-editing machinery within the cells. These damaged residues are deleterious to normal lens function if they accumulate, leading to loss of transparency and cataract formation. Recent research has identified biomolecules, many of which are antioxidants, that are active in preserving lens function by helping to remove or detoxify these residues, thus delaying damage to the bulk proteins essential to normal lens function.

In the normal course of ocular functioning, light and various biochemical processes damage lens proteins and other cell constituents that are essential for retaining the light-focusing function of the lens. In a young, healthy individual, the cell's protein-editing machinery quickly removes these damaged proteins. As we get older, the damaged proteins can accumulate faster than they are removed. The buildup of this material damages cellular components, interferes with normal functioning, and can lead to the opaque deposits typical of cataract. Dr. Allen Taylor's presentation at the symposium described work in identifying biomolecules that are active in preserving lens function; many

of these biomolecules are antioxidants. These biomolecules prolong and enhance the functioning of the protein-editing machinery, thereby preventing or delaying the damage to bulk proteins that causes cataract. The importance of antioxidants was a recurring theme at this symposium, not only for slowing the formation of cataract but also for slowing the progression of other ocular diseases, as described below.

Beyond the processes implicated in cataract formation, the symposium participants found this research intriguing for its potential in using the lens as a system for studying general aging and premature mortality. The layers of the lens represent different ages of lens cells within the tissue and can therefore be used to segregate zones of proliferating from nonproliferating tissue, i.e., young versus aged tissue. Effects of environmental exposure in stressing these layered, differential age zones of tissue can be studied. Thus, gerontologists and geriatric researchers, working with researchers on lens biochemistry and molecular biology, could use the lens as a window into processes vital to cell differentiation, development, and early senescence.

There is much evidence that exposure to the UV-B range of sunlight (280–320 nm) increases the risk of both cataract and corneal disease. Sunlight exposure and therefore risk of cataract formation is particularly high in specific occupational groups, such as fishermen, who are exposed to increased UV-B in the reflected glare from water. Worldwide, cataract continues to be a leading cause of preventable blindness, with about 1.5 million new cases each year, most of which occur in developing countries [8]. In the United States, cataract is still the leading cause of blindness among African Americans [4, p. 51]. Wearing proper sunglasses or even a brimmed hat greatly decreases exposure to sunlight and thus the risk of developing cataract.

8. Even nutrients normally thought of as "safe" can have deleterious effects. Simple sugars, for example, can damage the bulk proteins of the lens and compromise cellular editing. Maintaining adequate levels of omega-3 fatty acids appears to aid in the health of the lens as well as other ocular tissues. Thus, a prudent preventive strategy for cataract includes optimizing diet to prolong healthy cellular function. To support this preventive strategy, further work is needed to

examine systematically the potential of other common nutrients for adverse or beneficial effects on lens transparency.

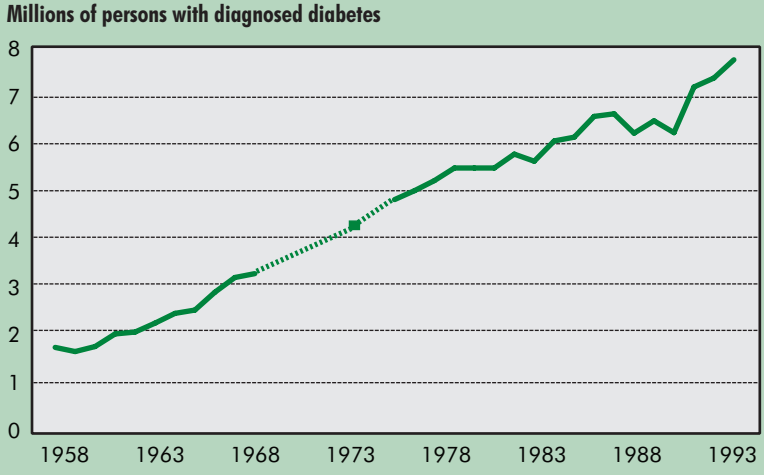
Overarching conclusion 2 noted the deleterious effects of a diet high in simple carbohydrates. Dr. Taylor's presentation included evidence for mechanisms by which overabundance of simple carbohydrates can damage bulk proteins in the lens and compromise the protein-editing capability that normally prevents accumulation of damaged proteins. Limiting the intake of simple sugars can reduce the rate of damage to lens (and retina) proteins, and there is no counterevidence to observations that diets lower in simple sugars are associated with healthier lens and retina conditions. Therefore, a diet designed to restrict the intake of simple carbohydrates is a reasonable part of a prudent preventive strategy for cataract and retinal damage, just as it is for systemic health consequences such as obesity and type 2 diabetes. As noted above in discussing conclusion 2, the benefits of a diet low in simple carbohydrates can be readily achieved through minor changes in dietary practice.

Nutrition, Environment, and Diabetic Retinopathy

9. Diabetic retinopathy is an important and rising cause of blindness in the United States and around the world. Systemic management of diabetes and prevention of retinopathy are perhaps the most effective approaches for reducing diabetic blindness in the future.

In his presentation on diabetic retinopathy, Dr. Rohit Varma stated that the scientific evidence for a nutrition-related risk factor for DR as a consequence of type 2 diabetes mellitus is overwhelming. Although genetic susceptibility plays a part in who does or does not develop type 2 diabetes and how soon it develops, the typical American diet—high in unneeded calories and particularly high in fats and simple carbohydrates—is the main culprit in the rapidly increasing prevalence of this disease in Americans of all ages (figure 2).

Figure 2. Prevalence of Diagnosed Diabetes in America



Source: [10, appendix 4-2, p. 63].

DR is a rising cause of blindness in America because of the increasing number of people whose lifestyle increases their risk of developing diabetes and because of the subsequent lack of strict control of blood sugar level after an individual develops type 2 diabetes. Knowledge of the necessity for a disciplined approach to control of blood sugar, along with the information on how to maintain that control, is currently the best hope for a strategy to ameliorate the national trend toward increased prevalence of DR and higher rates of progression to severe vision loss and blindness.

10. Knowledge of the risk factors for DR and diabetes in general is important in counseling patients and in devising therapeutic approaches.

(a) Diet control is a critical factor not only in managing the disease and its progressive vision loss consequences but often in preventing diabetes from developing.

(b) Early screening at appropriate intervals is key to the management of DR.

(c) The sociocultural environment is clearly important in development of type 2 diabetes and of DR in all forms of the disease. These sociocultural factors

have not yet been sufficiently investigated. Further research is needed into how these factors translate into barriers to ideal prevention and management of DR for the general population and for special populations.

Diet, exercise, and behavioral modification are all essential to the management of diabetes and control of blood glucose. Long-term control of blood glucose is critical in reducing the incidence and progression of DR. Elements of a holistic strategy to control diabetes, and thereby lessen the risk of progression to DR, include the individual patient's commitment to all of the following activities:

- Self-care (including diet)
- Seeking medical care, particularly including ophthalmic examinations on a regular basis
- Monitoring and control of blood sugar on an intraday basis (more frequent monitoring and control action than just once a day)
- Regular exercise

Because we have a good understanding of how these behaviors affect the course of the disease (DR and the underlying diabetes), they provide an evidence-based foundation for effective treatment.

In spite of recent and anticipated progress in developing therapies specific for DR (local therapies) with fewer toxicities or adverse effects than photocoagulation, preventive strategies should remain the single most important aspect of disease management. Future systemic approaches to preventing vision loss from DR—including slowing or halting progression of the disease—will target the achievement of a physiologically normal glucose/insulin homeostasis to manage the underlying diabetes. Local therapies for managing DR once it appears are likely to target fundamental disease mechanisms and will probably be synergistically combined. For example, patients will take a "cocktail" of several medications. Since diabetes is a chronic disease, sustained delivery platforms providing long-term dosing with pharmacotherapeutics will likely be an important facet of both systemic and local therapies.

Improvements in diagnostic technologies combined with focused educational programs will be critical for successful screening and early detection of disease and prevention of disease progression. Over the

past 5–7 years, several screening programs at the community level have been successful in detecting early-stage DR. Such programs are valuable for preventing progression of the disease from lack of diagnosis and for channeling affected individuals into the type of care they need for their disease stage. These successful programs constitute a major improvement in the public health outlook for preventing vision loss from DR. However, the relatively small scale of these community-level programs needs to be extended: for example, to citywide and statewide screening programs. The discussion following Dr. Varma's presentation highlighted a range of issues in translating the success of these smaller scale programs to larger populations.

The importance of sociocultural factors in type 2 diabetes and DR resulting from it is underscored by epidemiologic data on differences in prevalence among special populations, as in Dr. West's Arizona Proyecto Vision Evaluation and Research study of a Latino population and Dr. Varma's Los Angeles Latino Eye Study. In most instances, the principal risk factors distinguishing these special populations appear to be sociocultural—and hence environmental—rather than genetic. The data also point to substantial differences among sociocultural groups with respect to access to and acceptance of the health care system in which screening, preventive management, and stage-specific disease treatment are embedded. An effective public health strategy for DR (as for diabetes diagnosis and management) must recognize and work with these sociocultural factors, using them to advantage instead of allowing them to become barriers to health care. Directed research is needed not only to confirm and refine the epidemiologic data relating prevalence to specific sociocultural factors but also to develop, test, and translate into practice more-effective methods for working with groups differentiated by their sociocultural environments.

Nutrition, Environment, and AMD

11. AMD is a multifactorial disease in that both gene mutations and environmental factors contribute to the disease process. Data on gene-environment interactions

imply that the increased risk of AMD associated with some alleles may be ameliorated by healthy lifestyles. In general, since environmental factors are modifiable, maintaining a healthy diet and not smoking are still essential recommendations for those at increased risk.

The epidemiologic evidence reviewed in Dr. Caroline Klaver's presentation supports the view that genetic factors contribute more to AMD occurrence than do environmental factors. Nonetheless, since we do not yet have a way of modifying the genetic component of AMD risk, modifying the environmental factors that increase risk remains an important health care strategy. The preliminary data from recent studies imply that the deleterious effect of the gene variants (alleles) that increase risk for AMD occurrence may be at least partially ameliorated by healthy lifestyles. Environmental risk factors for AMD include smoking (a risk factor identified in many studies) and poor diet.

12. Due to differences in many published studies in design and outcome, the body of data on the effects of diet on AMD risk appears largely inconsistent at present. As in the case of cataract, additional rigorous studies are needed to determine the efficacy and safety of other dietary modifications.

The conflicts in the current data on diet are probably due to differences in study design, choices of outcomes to follow, and the lack of standardization in cut-off points for reference group and risk categories. In addition, study populations have typically been too small to provide adequate statistical power. The lack of consistent reference to recommended daily allowances hampers the interpretation of dietary values. Despite these disparities on dietary factors, consumption of fatty fish and high intake of certain antioxidants, such as vitamin E and the mineral zinc, appear to be beneficial [11, 12].

13. The AREDS results show that combinations of antioxidants can be effective in slowing AMD progression. These antioxidants may interfere with the pathways of programmed cell death (apoptosis) in the retina, which are associated with loss of photoreceptor cells in retinitis pigmentosa as well as AMD. A general intervention for these pathways of programmed cell death and photoreceptor loss should be

useful in preventing or slowing the progression of a range of diseases besides AMD—including retinitis pigmentosa, glaucoma, and even diabetic retinopathy.

The beneficial effects of a dietary supplement containing antioxidants and zinc for AMD patients with advanced AMD in one eye already were first reported from the Age-Related Eye Disease Study (AREDS) in 2001 [13]. Dr. Emily Chew reported in her presentation at the symposium that the 10-year follow-up with the AREDS participants is expected to show that the beneficial effects persist for the treatment group with advanced AMD. The 2001 report and subsequent AREDS reports noted that there were no statistically significant beneficial treatment effects of the tested supplements on early stages of AMD.

Nevertheless, a surprising number of patients with advanced AMD are not taking the AREDS supplement, while some people with no AMD or early-stage indications are taking the supplement in hope of preventing progression of their condition. Similar difficulty with translating research results into evidence-based care occurred with the earlier results from clinical studies of the efficacy of strict control of blood glucose levels on progression of DR. Even 10 years after the major DR studies, many diabetic patients were unaware of the studies' implications for managing diabetes to prevent or retard vision loss.

As part of the planned dissemination of the results from randomized controlled clinical trials, sponsors and study leaders should incorporate plans for conveying the implications for patient care (both practitioner care and self-care) to both practitioner and patient communities. Consideration should be given to metrics for how well this practical information has reached the key target audiences. The initial AREDS trial (AREDS1) and the new follow-up study (AREDS2) on other antioxidants such as lutein could be used as initial test cases for both dissemination methods and metrics for the success of those methods.

Continuation of randomized, controlled clinical trials of supplements is necessary to test new antioxidants and combinations for efficacy in mitigating the progression of not only AMD but other chronic, progressive eye diseases such as DR, glaucoma, and RP. Studies will also be

needed to evaluate gene-gene, gene-environment, and gene-nutrition interactions. Another promising direction is research and clinical trials to evaluate the role of inflammation and oxidative damage in both the pathogenesis and therapy of AMD. In particular, several gene variants associated with AMD occur in genes that code for proteins important in the immune system. This evidence for genetic susceptibility to AMD makes it critical to investigate the role of inflammation in causing or promoting AMD progression.

The discussion of Dr. Chew's presentation included the prospects for and diagnostic value of a confirmed serum test for markers of oxidative damage and inflammation in the retina. There is evidence that such biochemical markers may indicate the early stages of macular degeneration or other degenerative diseases of the retina. The participants agreed that evaluation of potential markers of inflammation and oxidation in the retina should be a supported research objective. Other areas of research that appear promising for early detection of retinal damage and determination of the potential value of nutrient supplementation to prevent or ameliorate it include the following:

- Methods of measuring supplement uptake (including active metabolites of the supplement) by a target organ or tissue, such as the retina–retinal pigment epithelium (RPE) complex
- Measurements of macular pigment (as a quantitative marker of retinal stress or damage)
- Capability to map metabolic activity in retinal tissues
- Use of high-resolution (spectral domain) optical coherence tomography to assess retinal integrity noninvasively

The discussion of Dr. Caroline Klaver's presentation on nutritional and environmental risk factors for AMD in relation to genetic susceptibilities also led to consensus on research objectives that should be pursued. First, to evaluate the true benefit of nutrients in lessening AMD risk, a meta-analysis is needed of all the prospective epidemiologic studies with follow-up that have used the same or similar methods. To avoid limitations of previous attempts at a meta-analysis, the nutrient cut-off points used

in this new analysis and in additional studies should be compared directly with the recommended daily allowance for each nutrient tested.

Second, the risks for early- and late-stage AMD need to be evaluated separately, to determine which nutrients may or may not be important for which outcomes. The objective should be to formulate and confirm better advice on the value of nutritional supplements or special diets than can be scientifically defended at present. For example, advice for individuals at increased risk because of genetic factors must go beyond encouragement to eat more fish and leafy green vegetables. An evidence-based nutritional strategy should be grounded in well-supported links from disease stage or risk to the quantities and concentrations of beneficial nutrients that should be in a recommended diet. The long-term goal should be a risk-lowering diet with specific nutrient intake levels, which can be recommended to patients at risk.

Third, smoking is an important and well-established risk factor for AMD, but more should be known about how the risk decreases after someone quits smoking. For instance, at the progressive stages of the disease, how much time is needed to benefit from quitting? Acquiring an adequate knowledge of how quitting lowers the risk profile for former smokers will require long-term prospective studies that cover all the AMD stages. This objective would also be aided by a meta-analysis, as described above, of results from prospective studies with follow-up.

Fourth, to maximize their scientific and clinical relevance, future epidemiologic studies on AMD should acquire data from the study population on genetic risk alleles. These data on genetic factors, both established and potentially of interest, should be acquired, documented, stored, and made accessible in data formats and with supporting contextual information (metadata) necessary to conduct subsequent data-mining analyses aimed at testing hypotheses that are formulated subsequent to the original study plan. For example, the symposium participants were enthusiastic about the efforts to acquire, document, and archive genetic samples and data from participants in AREDS1, AREDS2, and the Rotterdam Eye Study.

Nutrition, Environment, and Retinitis Pigmentosa

14. A small positive effect of vitamin A in slowing vision loss for some retinitis pigmentosa patients has been known for some years. However, the finding that combinations of antioxidants slow vision loss from RP in animal models is an exciting new development and should be explored systematically in both preclinical studies and clinical trials. If a nutritional supplement formulation is found to be beneficial in slowing the progression of photoreceptor loss in RP or other diseases of the retina, a study of the nutritional habits and potential nutritional deficits of populations in different geographical areas and of different cultural backgrounds will be important to undertake. Such a study would be valuable for addressing the following questions:

(a) Which special populations are likely to benefit from taking a nutritional supplement?

(b) Which supplement formulation is likely to be needed over an entire lifetime?

(c) Which special populations might not benefit?

Dr. Theo van Veen described the status of controlled clinical trials whose preliminary results indicate that nutritional interventions with vitamin A palmitate and increased consumption of fish rich in omega-3 PUFAs can slow the progression of RP in many patients. He also described new studies conducted in his laboratory and recent work by Dr. Peter Campochiaro at Johns Hopkins University that indicate the potential value of antioxidants in treating RP. In these studies, combinations of antioxidants appear to have a greater effect in slowing retinal degeneration in several rodent models of RP than does any single antioxidant.

The current thinking is that these antioxidant combinations work by scavenging reactive oxygen species that proliferate in the outer retina due to the high oxygen environment (hyperoxia) where rod photoreceptor cells have been degenerating. The early stages in the pathway that leads to rod photoreceptor loss in RP (e.g., specific gene mutations) differ from the initiating conditions for other diseases of retinal degeneration. However, this subsequent sequence of hyperoxia in the outer

retina, accelerated loss of rod cells, and then cone cells, resulting in eventual restructuring throughout the cross-section of the retina (not just the outer layer of photoreceptor cells), may be common to a number of diseases of retinal degeneration, including DR and AMD as well as RP. Although the initiating conditions would not be affected, more effective removal of reactive oxygen species in the outer retina could help slow disease progression toward severe vision loss. By reducing the formation of reactive oxygen species or accelerating their removal, the functional life of structural proteins and of enzymes that maintain protein quality can be extended.

The symposium participants agreed with Dr. van Veen's recommendation for systematic exploration of the use of combinations of antioxidants to intervene in these processes by which photoreceptor cell death accelerates toward vision-threatening endpoints. Among the candidate antioxidants that could be investigated for inclusion in an optimally effective dietary combination are (1) lutein and zeaxanthin, (2) suitable glutathione mimics, (3) α-lipoic acid, (4) vitamins A and C, (5) MnTBAP [manganese(III) 5,10,15,20-tetrakis(4-benzoic acid)= porphyrin], and (6) omega-3 PUFAs including docosahexaenoic acid (DHA).

If dietary management and supplementation are confirmed to increase the effective concentration of antioxidant species in the outer retina, then the next step toward cost-effective preventive care is to determine which antioxidants need to be enhanced or increased to optimize the antioxidant combination present in the retina. As has been shown in studies of vitamin A intake, the amounts of antioxidants in the diet depend on geographic and sociocultural factors. Dr. van Veen emphasized that, if a dietary supplement is going to be prescribed for the remainder of an individual's life, the cost-effective public health strategy is to know which supplements are needed for a particular dietary pattern. Therefore, systematic studies are needed of nutritional habits in different geographical areas.

Another reason for tailoring long-term dietary supplements to the optimal needs of a dietary pattern is the concern that high levels of a specific supplement may have deleterious effects. The symposium par-

ticipants discussed recent studies on the safety of high-dosage vitamin E supplements (400 IU per day or higher), which did not confirm the increased mortality risk reported in an earlier study. The controversy over vitamin E supplements, whichever way it is resolved, supports the need for further studies to establish safe maximum levels not only of vitamin E but of other nutrients that are advocated as health-improving dietary supplements. The general point is that increasing dietary intake of something that is beneficial at lower dietary levels may not only be ineffectual but could be harmful.

Crosscutting Issues

Two nutrition-related topics that the participants discussed at length are timely and relevant to most of the eye diseases considered during the symposium. One topic was the emerging importance to eye health and disease therapy of lipids, particularly PUFAs, and their bioactive products. The second topic was the general area of nonconventional therapies, or complementary and alternative medicine, particularly with respect to which claims for such therapies have or have not been confirmed by scientifically rigorous studies.

The Emerging Importance of Lipids and Their Bioactive Products

15. Evidence from the cellular and molecular biology of the normally functioning eye, together with evidence from the pathophysiology of some retinal diseases and from animal models of retinal degeneration, supports an important role for long-chain polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA), in photoreceptor physiology.

(a) Biologically active molecules derived from DHA, called docosanoids, appear to play significant roles in protecting retinal cells, including the photoreceptors, from the damaging effects of inflammation and programmed cell death.

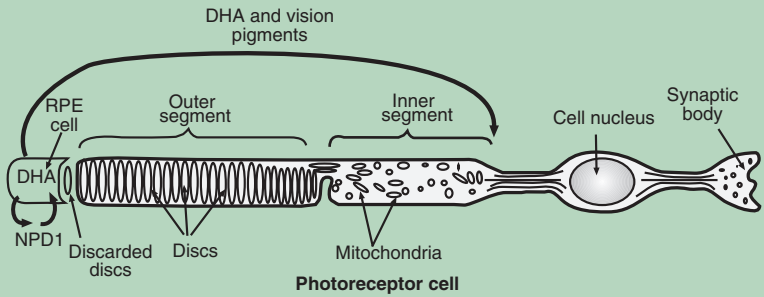
(b) DHA is a critical building block from before birth through early childhood. With more recent data on the ratio in breast milk of DHA (an omega-3 fatty acid) to omega-6 fatty acids, the ratio of DHA to arachidonic acid

in commercial infant formulas should be increased and new products that provide a dietary source of DHA in the second year of life should be developed.

The outer segments of photoreceptor cells have the highest DHA content of any cell type in the human body. Photoreceptor cells also retain their DHA tenaciously. The discs from which the outer segments are composed are shed, captured, and broken down into constituents by RPE cells, and replenished (figure 3). This renewal process is crucial for healthy visual functioning. During outer segment renewal, the RPE recycles DHA back to the photoreceptor cell's inner segment for reuse in forming new discs to add to the base of the outer segment. In this process, the oldest discs at the tips of the outer segments closest to the RPE cells are shed in small packets, which are ingested and digested by the RPE cells. DHA is returned to the photoreceptor cells to complete a metabolic loop. This loop of DHA conservation suggests that DHA is a critical component for normal functioning of photoreceptor cells.

The importance of DHA to the developing nervous system is highlighted by the fact that DHA constitutes 30–40 percent of the total fatty acids in the retina and in the brain—far higher than in other tissues.

Figure 3. Recycling of DHA and Vision Pigments



Note: DHA and vision pigments are recycled when outer segment discs of a photoreceptor cell are ingested by an RPE cell. The RPE cell also synthesizes NPD1 from DHA.

Source: Adapted from Dr. Nicolas Bazán, presentation to the Fifth Drabkin Symposium, June 2007.

DHA is a member of the omega-3 family of essential fatty acids. It can be made in the human body from dietary sources of linolenic acid (omega-3 fatty acids) or directly supplied in the diet as DHA. Sufficient dietary intake of DHA is critical during key stages of prenatal, neonatal, and early childhood development, when the organs that need DHA—retina, brain, and nerves—are growing rapidly. DHA-supplemented infant formulas enhance (1) maturation of retinal function, (2) visual acuity, and (3) overall neurological performance in preterm and term infants. The physiological value of DHA is also indicated by the high efficiency with which the central nervous system conserves it, including but not limited to the retinal outer segment renewal process described above. Several observational studies indicate benefits for systemic and ocular health from a diet that is lower in simple carbohydrates than the typical American diet but higher in intake of dark-meat fish, which is a good source of omega-3 fatty acids such as DHA and eicosapentaenoic acid. Table 2 lists some important dietary sources of these fatty acids for nursing mothers, weaned children, and adults. In adults, prolonged dietary deprivation of omega-3 fatty acids is required to reduce DHA content in

Table 2. Dietary Sources of DHA

Food	DHA (mg)
3 oz pink salmon filet, baked/broiled	638
3 oz pink salmon, canned	589
3 oz white tuna (albacore), canned in water	535
3 oz smoked salmon (lox)	227
3 oz blue crab, steamed	196
3 oz light tuna, canned in water	190
12 large shrimp, steamed	95
3 oz tuna salad	47
2 pieces chicken (drumsticks), fried in flour	39
2 large eggs, hard-boiled	38

Source: [14]; accessed June 7, 2006.

the body to the point that functional impairments occur. This metabolic retention of DHA demonstrates the tenacity with which the body, especially the retina, retains this cellular building block and precursor of critical bioactive docosanoids: metabolically produced derivatives of DHA.

There is long-term evidence that DHA levels in the blood are decreased in some cases of RP. Rodents with rhodopsin mutations that are homologous to mutations in some types of human RP also have decreased levels of DHA in their photoreceptors. However, the evidence for a DHA role in human RP is not consistent, even for specific RP phenotypes. Thus, the relevance of low serum DHA to human RP pathology has yet to be clarified. Studies of AMD suggest an inverse relationship between diets high in DHA and AMD risk.

At the symposium, Dr. Nicolas Bazán described his work with docosanoids, which appear to play important roles in healthy functioning of the retina and other ocular tissues. Metabolic pathways for enzyme-mediated oxygenation of DHA lead to the synthesis of docosanoids, which act as intercellular and intracellular messengers in the retina and as neuroprotective mediators. For example, neuuroprotectin D1 (NPD1) can counteract cellular changes that are triggered by multiple factors and that would otherwise lead to inflammation and cell damage.

NPD1 is a DHA-derived bioactive molecule, synthesized within the body; it modulates signaling that promotes cell survival. NPD1 appears to play an active role in neuroprotective signaling responses to oxidative stress and neurodegeneration. Dr. Bazán's group has found that regulation of NPD1 synthesis by neurotrophins (growth factors active in the nervous system) and other factors may redirect cellular fate in response to injury or neurodegeneration away from apoptosis and toward successful cell survival and aging. In addition, NPD1 promotes homeostatic regulation of cell integrity during photoreceptor cell renewal. It protects the RPE from damage due to oxidative stress and the presence of A2E, a byproduct of outer segment disc recycling that becomes toxic if it accumulates. Therefore, NPD1 and the pathways in which it is active provide a potential target for therapeutic interventions employing the natural neuroprotection and/or neurorepair functions of this DHA derivative.

The importance of DHA and another PUFA, arachidonic acid (ARA), for infant brain development has emerged during the past 30 years and more. DHA levels in the brain increase substantially from the third trimester of fetal development through at least 2 years of age. Throughout this time, the brain is developing rapidly. Infants fed formula that is not fortified with DHA have lower tissue levels than breast-fed infants or those fed with fortified formula. Infants fed with formula fortified with DHA and ARA have significantly better visual acuity and cognitive development than infants fed unfortified formula. At the symposium, Dr. Eileen Birch used these results and others from her work to emphasize the conclusion that providing adequate supplies of DHA to the developing nervous system is critical from the time an infant is in utero through early childhood.

The current levels of DHA fortification in infant formula are based on studies from the 1980s and early 1990s showing that concentrations greater than or equal to 0.25 percent DHA were associated with improved visual and cognitive function. However, these studies did not establish an optimal concentration for infant formula or an optimal level for DHA supplementation in the maternal diet (for both prenatal and breast-feeding infants). The formulation currently used in the United States has a ratio of DHA to ARA of 1:2. This ratio was based on samples of North American human milk tested prior to 1992, when the American diet had a higher proportion of omega-6 PUFAs (ARA is an omega-6 PUFA) to omega-3 PUFAs than is typical in many less-industrialized settings, where the diet is higher in DHA and other omega-3 PUFAs. ARA and its metabolites are proinflammatory lipidic mediators that may play a role in allergic response and asthmatic response. High intake of omega-6 PUFAs is also a risk factor for such chronic diseases as coronary artery disease, hypertension, and immune/inflammatory disorders. Thus, these newer lines of evidence support increasing the ratio of DHA relative to ARA.

Nonconventional Therapies: What Works and What Doesn't

16. Substantial numbers of patients with chronic eye disease use nonconventional treatments to complement, or as an alternative to, treatment prescribed by a

physician. The scale of such use is great enough to raise significant issues of safety and efficacy—including the cost of expenditures on these treatments. In light of the substantial use of nonconventional treatments by Americans suffering from or concerned about eye disease, the symposium participants supported the following conclusions:

(a) Although many factors are likely to contribute to this substantial interest in and consumer expenditure on nonconventional treatments, there appears to be no systematic study of why people turn to them.

(b) Given this societal context, health care providers and educators should learn if a patient is using a nonconventional therapy and be informed about the scientific evidence (or lack thereof) for both efficacy and potential harm associated with its use as an alternative or complement to conventional treatment. Potential harm includes any direct negative (e.g., toxic) effects of the agent or procedure and the economic cost of ineffectual treatments, as well as the consequences if the patient ceases to follow a conventional therapeutic regimen. The symposium participants suggested that a provider's responsibility begins but does not end with informing the patient about any safety and efficacy issues related to a nonconventional therapy. Unfortunately, health care providers or marketers often fail to carry through on this responsibility.

(c) As an example, in the treatment of glaucoma, no scientifically sound evidence exists to support the claims made for a variety of nonconventional therapies, including the use of vitamins, herbs such as gingko biloba and bilberry (purported to be beneficial antioxidants), acupuncture, meditation, or special diets. In distinct contrast to these therapies that have inconclusive or no supporting evidence for efficacy, both short-term and moderate long-term exercise have been shown to reduce intraocular pressure. Thus, exercise should be considered as useful complementary therapy to accompany the standard medical treatments for glaucoma.

The presentations by Dr. Sayoko Moroi and Dr. John Hetherington reviewed the scientific evidence for the safety and efficacy of herbal medicines and dietary supplements that are advocated informally and commercially to protect vision or to treat chronic blinding diseases. Retail market distribution, advertising, and sales, as well as patient sur-

veys, demonstrate the substantial economic expenditures being made on nonconventional therapeutic products and services.

The symposium participants discussed a range of reasons why people use CAM: (1) They are not satisfied with the conventional treatments accepted in allopathic medicine. (2) They may not have access to mainstream care and conventional treatments for socioeconomic or health care infrastructure reasons. (3) Conventional treatments for their condition are unavailable or have been exhausted without success. (4) They may not believe in the efficacy or safety of conventional treatment, or they may believe that their nonconventional treatment is safer or more effective.

For comparison, an NCCAM study [15] using data from the 2002 National Health Interview Survey reported the following respondents' reasons for use of CAM for all conditions (respondents could give multiple reasons):

- 55 percent of the responding CAM users said that they thought the CAM therapy *combined with* conventional medical treatment would help.
- 50 percent said that they thought the CAM therapy would be interesting to try.
- 28 percent said they believed that conventional medicine would not help their condition.
- 26 percent said that the CAM therapy was suggested by a conventional medicine practitioner.
- 13 percent said that conventional medical treatments were too expensive.

Whatever their reasons, Americans are incurring large out-of-pocket expenses to purchase CAM products. A survey that compared alternative-medicine use (for all purposes, not just vision-related ailments) by Americans in 1991 and 1997 found an increase from 34 percent to 42 percent in use of at least 1 of 16 alternative therapies [16]. The study's estimate of 629 million visits to alternative medicine practitioners in 1997 exceeded the total visits that year to all U.S. primary care physicians. The estimated $12.2 billion paid in out-of-pocket expenses for alternative medicine professional services exceeded the 1997 out-of-

pocket costs for all U.S. hospitalizations This study, which is still cited by the NCCAM [15], estimated the total out-of-pocket costs for alternative medicine products and services to be at least $27 billion—comparable to the projected costs that year for all U.S. physician services [16]. A later study found that Americans spent $1.4 billion in 2000 just on herbal supplements [17].

Dr. Moroi reviewed the literature on CAM use by patients with eye disease, as well as the scientific evidence for safety and efficacy of the more widely used CAM therapies for the chronic diseases discussed at the symposium. CAM therapies for all major chronic eye diseases were not addressed in depth at the symposium and therefore are not covered at the individual disease level in this report. Glaucoma is used here to illustrate how the overall widespread use of CAM in America relates to eye disease because much of the available information in this area is about CAM use for glaucoma.

A 2002 study reported that 5.4 percent of patients in two large urban glaucoma practices were using CAM for their glaucoma. The types of CAM they used included megavitamin therapy (63 percent of CAM users), herbal therapy (57 percent), exercise (24 percent), and diet modification (22 percent). Thirty-two of the 54 respondents who used CAM (59 percent of CAM users) reported using more than one type. Meditation, acupuncture, faith healing, and homeopathic remedies were each used by one respondent [18]. Dr. Moroi suggested that some off-label uses of conventional medications and devices should be considered as CAM therapies until evidence for their efficacy in the additional use is established.

There are challenges in investigating the efficacy and safety of non-conventional therapies. Along with the standard challenges of clinical trial and cost, it is essential to ensure product (or device) quality and reliability of source. Investigations of nonconventional therapies must adhere to the fundamentals of sound scientific research, including hypothesis-driven questions and methods based on appropriate approaches. These approaches may be biological, biochemical, pharmacologic (pharmacokinetic and pharmacodynamic), genetic, pathological, epidemiologic, and/or statistical.

Given this societal context of widespread use of CAM and challenges in establishing therapeutic value, the symposium participants agreed that health care providers and educators have a responsibility to be aware if nonconventional therapies could potentially harm a patient. Potential harm includes the economic cost of ineffectual treatments. This responsibility begins, but does not end, with informing the patient of safety issues.

Health care providers must remember to interpret results from a clinical trial in the context of the individual patient. As the report from the Fourth Drabkin Symposium concluded, special populations within a diverse population may well experience disease prevalence and pathophysiology with substantial differences from the experience of the general population [4]. In recommending for or against a CAM option—or even a conventional treatment option—practitioners should consider the possibly relevant differences, environmental as well as genetic, age, and gender differences, for the individual patient and should not treat the patient based on an "average result." On this point, Dr. Moroi noted that epidemiologic results typically include outlier experiences as well as statistical norms. In addition, many patients treated by practicing ophthalmologists do not fit the inclusion/exclusion criteria that were used in clinical trials on which standard care patterns are based.

Dr. Hetherington reviewed the published scientific evidence on the safety and efficacy of CAM therapies specifically for glaucoma. He reported that no scientifically sound evidence exists to support the claims made for a variety of these therapies, including vitamins, herbs such as gingko biloba and bilberry (touted as beneficial antioxidants), acupuncture, meditation, or special diets. Dr. Hetherington drew the following conclusions from his review of the literature:

- There is no proven value of vitamins as glaucoma therapy.
- To date, marijuana and several specific constituent compounds (cannabinoids) have shown no proven effect on low-tension glaucoma or chronic open-angle glaucoma. However, testing of marijuana derivatives is ongoing.
- No objective evidence supports any value of herbal supplements, including gingko biloba and bilberry, for treating glaucoma or

lowering intraocular pressure (IOP). Antioxidant properties have been claimed for gingko biloba and bilberry, and these properties are sometimes suggested as the basis for a positive effect on glaucoma.

- There have been no studies showing a lasting significant effect of diet, meditation, or acupuncture on glaucoma.

In distinct contrast to these CAM therapies that have no supporting evidence beyond anecdote, both short-term and moderate long-term exercise have been shown, in studies using accepted methods, to reduce IOP. Moderate long-term exercise has been associated with substantial IOP reduction that lasts up to 3 months after the exercise regimen ends.

2. Summary of Presentations

Nutrition, Environment, and the Eye

Dr. Sheila West

Dr. West began this introductory presentation on the theme of Fifth Drabkin Symposium with some caveats for interpreting research on nutrition as a factor in eye disease. Speaking in general terms, nutrition is the process by which we assimilate food and use it for growth, liberation and utilization of energy, replacement of tissues, and generally maintaining health. Because nutrition occurs through a complex process consisting of multiple stages—from the intake of food, digestion in the gut, and absorption into the bloodstream, to assimilation into tissues and finally excretion from the body—the presence of nutrients at an earlier stage may not mean the same forms or concentrations of nutrients are present in the tissues where disease-relevant events occur. What is eaten, for example, is not identical to what gets absorbed into the bloodstream after digestion. And the concentration of a nutrient in the systemic circulation should not be assumed to be the concentration present in ocular tissues. Furthermore, if a specific nutrient is needed or beneficial for healthy tissue activity, a lack of that nutrient in the food we eat at a given time may not mean the tissue is in a malnourished state. There may be ample body stores of the nutrient, or it may be synthesized from other nutrients within the body.

Studies of the role of human nutrition in disease therefore must deal with both biological issues and measurement issues. The biological issues

include, in addition to distinguishing between intake levels and tissue levels of a nutrient of interest, characterizing the full range and amount of nutrients in foods, particularly micronutrients that may have unrecognized roles. Of particular importance is the caveat that a supplement formulated to test the effect of a natural food source in a nutrition study may not provide all the same nutrients that the natural food provides. Another biological issue is that many of the processes and thresholds that influence the body's ability to maintain a healthy, functional balance (homeostasis) are only partially understood; others may still be unknown. The lower and upper boundaries, or thresholds, of the range within which the body can maintain homeostatic balance are critical. Below a lower threshold, a nutritional deficiency may develop; above an upper threshold for nutritional homeostasis, toxic effects may arise. Within the homeostatic range, none of the signs of deficiency or toxicity are likely to occur. Understanding this range of homeostatic balance is important for health care interventions.

An important measurement issue for nutrition studies is the validity and reliability of questionnaires used to gather data on subjects' diet and nutrition intake. This issue becomes particularly relevant when a questionnaire is used as a data collection instrument for a society or a culturally distinctive special group that differs from populations in which it has already been rigorously tested, calibrated, and validated. Physical measurements such as levels of nutrients and markers in serum or tissue can correct for deficiencies in nutrition-intake questionnaires, but in most study protocols these measurements are taken too infrequently, relative to the daily routine of subjects, to give a complete and reliable picture of effective nutrient concentration over the entire course of the study. This sparseness of data on effective nutrient concentrations, particularly in tissues of interest to disease initiation or progression, limits the certainty with which conclusions can be drawn relating long-term nutrient intake, tissue levels of the nutrient, and disease-relevant effects.

Variations on these biological and measurement issues arise in trying to study other environmental factors as well. For example, how much ultraviolet (UV) radiation from sunlight reaches tissues at various depths from the front surface of the cornea back to the different layers

of the retina? For many environmental factors of potential interest, the sources of exposure may not be delineated well enough to quantify levels of exposure and duration of exposure, both of which are important for investigating a dose-response relationship with implications for a particular chronic eye disease. The problems of limited knowledge of homeostatic processes, including the lower and upper thresholds of healthy functioning, apply to other environmental factors in much the same way that they apply to nutrients. Using a questionnaire to assess exposure levels and durations raises the same kinds of cross-cultural issues that apply to food intake questionnaires. For environmental exposures, the lack of a good marker for quantifying exposure dose often limits the ability to use physical measurements to complement and validate questionnaire data.

These caveats on studies of nutrition and environmental exposure should be kept in mind as one sifts through the results from such studies, Dr. West concluded. In evaluating the reasonableness and conclusiveness of inferences drawn by a study's author(s), or in drawing our own inferences from a study, we need to think about the potential constraints of the methodology used.

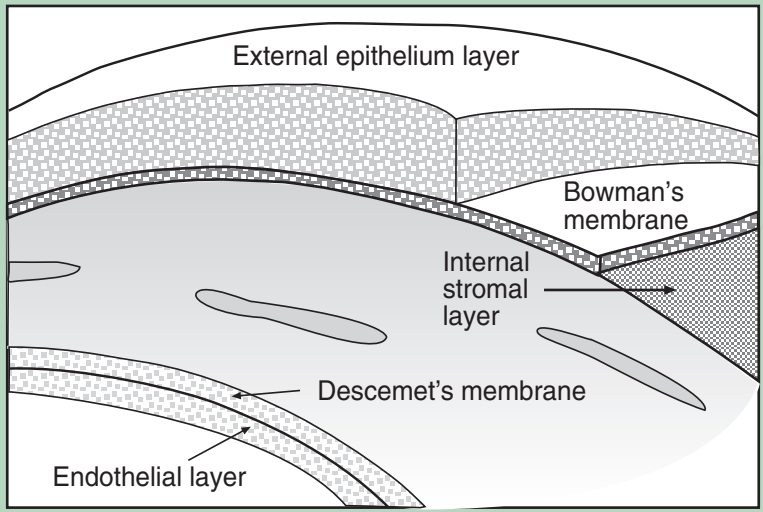
Nutrition, Environment, and Corneal Disease

Dr. Sheila West

Dr. West's presentation on corneal disease highlighted three topics: what is known about the role of vitamins and other nutrients in corneal health, homeopathic medicines promoted for corneal health, and the effects on the cornea of exposure to sunlight. She began with an overview of the layered structure of the cornea, shown schematically in figure 4. These layers work together to perform the cornea's multiple functions and maintain the transparency essential to its role in vision.

The external layer of epithelial cells is in direct physical contact with the external environment. Dr. West stressed the functional importance of the tear film that normally coats this outer epithelial layer. The thickest layer of the cornea is the internal stromal layer, which consists

Figure 4. The Multiple Layers of the Cornea



of parallel layers of collagen, important to both the clarity and flexibility of the cornea. A thin monolayer of endothelial cells on the inner side of the stromal layer prevents fluid from the aqueous humor below the cornea from getting into the stromal layer, which causes it to swell and become opaque. It also pumps fluids out of the inner corneal layers. Bowman's membrane separates the external epithelial layer from the stromal layer, while Descemet's membrane separates the stromal layer from the endothelium.

In conjunction with the ocular lens just beneath it, the cornea focuses light passing through it on the retina. To form a clear image on the retina, the cornea must remain transparent. Because the cornea has no internal blood vessels, which would interfere with transparency, its multiple layers must get all their nutrients from the aqueous fluid behind it, via the inner endothelium, or from the tear film on its external side. The fluid of the aqueous humor contains vitamins, glucose, and proteins needed by corneal cells. The tear film supplies oxygen and other essential compounds, as well as mucins for lubrication of the cornea's external surface.

The Role of Vitamins and Other Nutrients in Corneal Health

Vitamin A deficiency is the nutrient deficit most widely associated with diseases of the cornea. Although no longer a public health concern in the United States, Vitamin A deficiency continues to cause childhood blindness in Africa, south and southwest Asia, and some regions of the Middle East and South America (see figure 1, p. 21). The body does not synthesize vitamin A, although it does hold onto the vitamin A derived from ingesting the precursor compound, pro-vitamin A, or related carotenoids. Vitamin A is stored in the liver until needed elsewhere in the body, and normal metabolic processes retain and preserve it, typically recycling it for continued use.

Despite the body's efforts to conserve whatever vitamin A it acquires, retention is not perfect, and some dietary replenishment is essential, particularly during infancy and childhood development. In its early stages, vitamin A deficiency causes xerophthalmia, an abnormal dryness of the conjunctiva of the eye and the cornea. In a rural village, children displaying xerophthalmia are often a marker of vitamin A deficiency throughout the community. If the cornea has become opaque due to xerophthalmia and the condition is not relieved immediately by treatment with vitamin A, it can progress rapidly to corneal ulceration: essentially a hole growing into the layers of the cornea. If the corneal ulcer grows all the way through the cornea to the endothelial layer on the inner side, the result is keratomalacia: wholesale destruction of the cornea and extrusion of the contents of the eye through the hole that is left. Keratomalacia is a condition of total and irreversible blindness in the affected eye. Thus, rapid treatment of the earlier xerophthalmia stage is critical for avoiding this catastrophic outcome.

Breastfeeding of newborns typically replenishes the vitamin A stored in their liver. Routine consumption of foods containing vitamin A or precursor compounds is generally adequate protection against xerophthalmia. These foods include mango, papaya, eggs, cooked greens, carrots, and dairy products. Some infectious diseases such as measles can exacerbate cases of borderline vitamin A deficiency.

Where dietary sources are not adequate, a simple vitamin A supplement taken several times a year can adequately replenish the body's

store of vitamin A. Thus, the vitamin A supplementation programs conducted by UNICEF have been effective and efficient means of improving public health and preventing blindness. The cost of the more than 500 million capsules distributed annually in developing countries is only about 2 cents per capsule. One capsule every six months is generally sufficient. Where dietary sources are inadequate, UNICEF recommends providing vitamin A supplements to both children and nursing mothers. Food fortification programs are used in the United States and other countries to ensure adequate intake of vitamin A and precursors. The food chosen to be fortified is based on cultural preferences of the population at risk. In the United States, for example, milk and ready-to-eat cereals are fortified. In Guatemala, sugar is fortified, and in the Philippines, MSG (monosodium glutamate) is fortified.

With respect to other vitamins, Dr. West reported that her review of the medical literature did not turn up clear evidence of important associations with corneal disease. The aqueous fluid that fills the chamber between the cornea and the lens has a 26-fold higher concentration of vitamin C than the general level in the body. However, she found no reports in the literature of an effect of vitamin C deficiency on corneal health. Animals deprived of vitamin C have some changes in corneal cells, but none of the results from epidemiologic studies suggest that vitamin C protects against acute or chronic corneal diseases, such as those related to exposure to UV light. For vitamin E, one study in rats suggested that it protects against some of the consequences of vitamin A deficiency. However, Dr. West thought the clinical significance of this protective effect was unclear.

For a different category of essential nutrients, polyunsaturated fatty acids (PUFAs), there is suggestive but inconclusive evidence of potential for treating a corneal ailment commonly called "dry eye."[1] Dry eye, which afflicts more than 10 million Americans [19], is characterized by inadequate tear production, redness of the conjunctiva, and itching or burning sensations on the front of the eye due to abrasion by the eyelids.

[1]The medical name for dry eye syndrome is keratoconjunctivitis sicca.

Often in dry eye, filaments of epithelial cells peel off from the surface of the cornea.

In the 1980s, patients with an autoimmune disease called Sjogren's syndrome were reported to have their dry eye symptoms (which are often severe with this disease) alleviated by topical treatment with oil from the evening primrose plant. This oil contains the omega-6 PUFAs linoleic acid and γ-linolenic acid. In a more recent clinical trial, eye drops containing both of these omega-6 PUFAs were applied twice daily to treat 40 Sjogren's syndrome patients. The treatment group reported significantly fewer symptoms and had significantly less corneal damage than did the control group. However, other markers of dry eye—namely, tear breakup time and tear basal secretion rate—did not change with treatment [20].

As part of the Women's Health Study, 32,470 women aged 45 to 84 years completed self-report questionnaires, which included questions on diet and on dry eye symptoms. Within this group, 4.7 percent reported having dry eye symptoms. The researchers estimated dietary intake of omega-3 and omega-6 PUFAs from the diet information and used regression analysis to estimate the odds ratios for the association of PUFA intake with dry eye symptoms [19]. Based on this analysis, the omega-3 PUFAs were slightly protective for dry eye, but the association was statistically significant only in the group with the highest intake levels of omega-3 PUFAs. Although no statistical correlation, negative or positive, was found for omega-6 PUFA intake and dry eye symptoms, the analysis found an increased risk of dry eye for a diet with a high ratio of omega-6 to omega-3 PUFAs. (The odds ratio was 2.5 for a 15:1 ratio of omega-6 to omega-3 PUFAs compared with a 4:1 ratio.) In discussing their results, the researchers noted that a lower intake ratio of omega-6 to omega-3 PUFAs favors the metabolism of γ-linolenic acid to prostaglandin E1, which is anti-inflammatory, rather than to prostaglandin E2, which promotes inflammation [19].

Dr. West noted that the methodology for estimating the levels of dietary PUFAs in the Women's Health Study is subject to the caveats about nutrition questionnaires that she raised in her introduction to the symposium theme. She agreed that the two recent studies suggest an

association between PUFA intake and dry eye that should be followed up more thoroughly. Nevertheless, she cautioned, a popular health newsletter jumped on these very preliminary results and in 2006 advocated that women with dry eye eat more fish as a source of omega-3 PUFAs [21].

With respect to scientific studies of nutrients other than vitamins and PUFAs, a study of magnesium-deficient rats showed changes in the corneal epithelium but no striking consequences. Riboflavin deficiency was a concern in the 1950s, and studies from that period described cases of corneal vascularization with photophobia that were readily reversed when patients were given riboflavin. Animal studies have reported clouding in the stroma layer under conditions of riboflavin deficiency. With food fortification of cereals and flour since 1943, riboflavin deficiency has become rare in the United States.

Homeopathic Medicines Promoted for Corneal Health

Beyond the scientific literature, Dr. West said, many foods and supplements are being promoted as health treatments for dry eye and other ocular ailments. She described two products—one containing hyaluronic acid, the other a mixture of plant extracts—that are currently marketed as contributing to the health of ocular tissue including the cornea. For none of the ingredients in these products could Dr. West find any supporting evidence in the scientific literature for positive effect in treating dry eye syndrome. This marketing of products with unsubstantiated claims, Dr. West commented, illustrates that almost anything will be tried by people with dry eye, for which conventional treatment often provides inadequate long-term relief.

Other Environmental Effects on the Cornea: Sunlight and Refractive Surgery

The cornea absorbs most of the UV light in sunlight. Specifically, the cornea absorbs most wavelengths from 280 to 400 nm; shorter wavelengths (higher energy UV) are absorbed by the atmosphere, while the longer wavelengths from 400 nm to 700 nm, which constitute visible light, are transmitted to the retina where they stimulate photoreceptor cells.

The UV-B region (280–320 nm) and the UV-A region (320–400 nm) are the most biologically harmful portions of sunlight that normally reach the Earth's surface. Acute exposure to UV-B light reflected off snow can cause snow blindness (acute photokeratitis), a painful inflammation of the cornea accompanied by light sensitivity. Fortunately, it usually dissipates overnight. Long-term exposure to UV light can cause more lasting damage to the cornea and other exposed ocular tissues. Climactic droplet keratopathy occurs in areas with extended exposure to sunlit snow and afflicts fishermen who are exposed for long periods to UV light reflected off water, such as the watermen of the Chesapeake Bay. This condition is relatively rare even in populations with long-term exposure, so other factors appear to affect susceptibility.

Long-term exposure to high levels of UV-B light also increases the risk of pterygium, a thickening on the surface of the conjunctiva, usually at the inner (nasal) side of the eye. Pterygium can lead to obstructed vision if it grows out over the cornea. Factors other than UV exposure appear to be important here as well, such as exposure to certain viruses including the human papilloma virus.

Because the cornea is exposed to sunlight and absorbs the UV portion, the potential for damage is evident, Dr. West said. A simple solution is to wear UV-impervious sunglasses. Existing "sun-safe" public health campaigns, which currently focus on skin damage and the risk of skin cancer, could easily be expanded to warn of the risk of eye damage and encourage people to wear sunglasses.

Another potential environmental risk to the cornea that Dr. West discussed is the cultural trend toward refractive surgery as a way of correcting visual acuity without corrective lenses. In this procedure, a laser is used to score and reshape the surface of the cornea. The commercial entities that promote refractive surgery have made little or no provision for postmarket follow-up of those who undergo the procedure, even though it causes irreversible changes in the internal layers of the cornea. To highlight the potential public health consequences if long-term problems begin to appear in the treated population, Dr. West described refractive surgery as "the largest natural experiment in intentional cornea damage ever performed."

Post-Presentation Discussion

Dr. West was asked which questions about indicators of dry eye syndrome (for example, self-reported symptoms) should be included in questionnaires used for epidemiologic studies of eye health. After describing some of the approaches that have been used, Dr. West said the biggest problem is differentiating among types of dry eye. For example, there is a clear reason why omega-6 PUFAs would be protective for subjects with Sjogren's syndrome, but it is unclear why omega-3 PUFAs would be protective. In some studies, subject self-reports of dry eye symptoms could be validated with clinical evaluation of their condition. In the Salisbury Eye Study, Dr. West and her team found that some subjects were experiencing dry eye symptoms due to medications they were taking (iatrogenic dry eye). The symposium participants discussed the possibility of using the medications data that will be collected for the Age-Related Eye Disease Study 2 (AREDS2) clinical trial to identify cases that were likely to be iatrogenic dry eye.

Dr. Nicolas Bazán noted that he would provide further information on the relationship between omega-3 PUFAs and dry eye in his presentation. He gave some highlights of recent work with topical application of omega-3 PUFAs. (See the summary of Dr. Bazán's presentation, p. 109.) On the issue of refractive surgery, he noted that refractive surgery in animal models has caused damage to the corneal nerves that requires 2 to 3 years to heal. Application of omega-3 PUFAs appears to promote regrowth of corneal nerves after refractive surgery. He agreed with Dr. West on the importance of further work to clarify and validate the effects of PUFAs on the anterior segment of the eye, including the cornea and lens.

The participants discussed the potential significance of the ratio of omega-3 to omega-6 PUFAs and how that ratio might be estimated in terms of dietary patterns, rather than attempting to collect data on specific nutrients. Omega-3 PUFAs, according to Dr. Bazán, are concentrated in the photoreceptors of the retina and in the central and peripheral nerves, with much lower concentrations in other tissues. This distribution differs markedly from the distribution of the major omega-6 PUFA, arachidonic acid (ARA), and the processes by which the dietary

intake of PUFAs is sorted, metabolized, and distributed are incompletely understood. Dr. West related this point to her opening caveat that neither the concentrations nor the ratios of PUFAs in the diet may be reflected in the concentrations in tissues of interest.

Dr. Sayoko Moroi commented that iatrogenic dry eye is a concern for the pharmaceutical companies that market eye-drop medications to lower the intraocular pressure (IOP) of glaucoma patients. Antihistamines and antidepressants can also cause iatrogenic dry eye.

Dr. Caroline Klaver asked about the clinical evidence for prescribing high doses of vitamin C for patients with corneal ulcers. Dr. West replied that her literature review for the symposium had not included treatment for corneal infections, which in her experience are the primary cause for most corneal ulcers in developed countries. Dr. Allen Taylor added that the principle of such treatment would be that the vitamin C enhances repair of collagen. How much of the ingested vitamin dose reaches the cornea is a question, but the alternative of direct topical application to the eye could be painful. Dr. Moroi described, as an example of this approach, a homeopathic eye drop that a patient of hers was using to try to decrease cataracts. Also, other homeopathic medicines advertised for corneal health contain vitamin C. The participants discussed the significance of the normally high level of vitamin C in the aqueous fluid, which would be the natural route of supply to the cornea. In response to Dr. Gerald Chader's question on the biochemical pathway in the cornea that is affected by vitamin A deficiency, Dr. West said that goblet cells (epithelial cells that secrete the mucins found in the tear film) are depleted. Dr. Bazán commented that the retinoids, such as vitamin A, have roles as regulators in many body tissues. For example, vitamin A is a crucial modulator in the liver. The detailed molecular biology of the inducer and activator roles played by retinoids is still emerging.

Dr. John Hetherington described the frequent cases of keratomalacia he had witnessed during a recent visit to Afghanistan. A large number of patients were already totally blind at ages from 5 to 13 years old due to the lack of vitamin A in their subsistence diets. Dr. West said that diseases of vitamin A deficiency can be expected to increase substantially in any war-torn region with large refugee camps. Delivery of vitamin A

supplements or of vaccine to prevent measles becomes difficult to impossible in such circumstances, increasing the risk of blindness for refugee children.

Nutrition, Environment, and Cataract

Dr. Allen Taylor

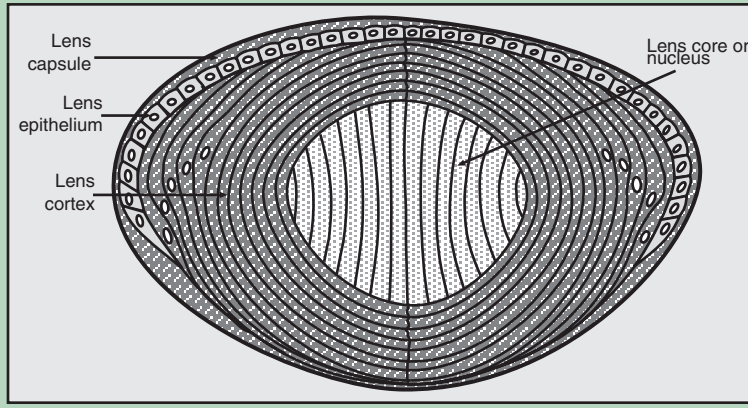
Dr. Taylor's presentation highlighted what is known about the biochemistry of cataract formation and the influences on these processes of important dietary factors such as antioxidants and carbohydrates. One objective of his talk was to stimulate interest in new ways to apply established biochemistry to the task of answering current questions about how cataracts form.

The Biochemistry of Cataract Formation

Along with the cornea, the function of the crystalline lens of the eye is to focus visible light on the retina to form an optical image. To do this well, the lens should remain clear and flexible, but with age it tends to become yellow or brown (brunescent), as well as becoming clouded from the increasingly opaque deposits whose endpoint is cataract. Cataract is typically a disease of aging. Within two decades, as the average age of Americans increases, cases of cataract and age-related macular degeneration (AMD) will increase by 50 percent [6]. Nevertheless, Dr. Taylor believes that processes that naturally keep the lens healthy in younger people can be enhanced to slow or halt cataract formation in older people.

The lens is made up of a single type of cell, but the cells differ in important ways depending on the metabolic zone in which they reside (figure 5). Lens cells close to the epithelial layer around the outside circumference of the lens are compact and contain all the organelles of a typical cell. In the cortex region, the cells are elongated into fiber cells, which produce highly specialized proteins called crystallins. Cortex cells retain their organelles, including the cell nucleus. The fiber cells in the core (or nucleus) of the lens, which are the oldest cells in the lens, have

Figure 5. Fiber Cell Structure of the Crystalline Lens



lost their cell nuclei and other organelles to become long, optically transparent fibers packed with crystallin. Losing the cell nucleus removes a barrier to transmission of light, but it also removes the normal machinery for repairing cell damage. Although the biochemistry that produces cataract-forming deposits is probably similar throughout the lens, the repair mechanisms in the nucleated cells of the cortex differ from the repair mechanisms for the genetically and metabolically quiescent cells of the core.

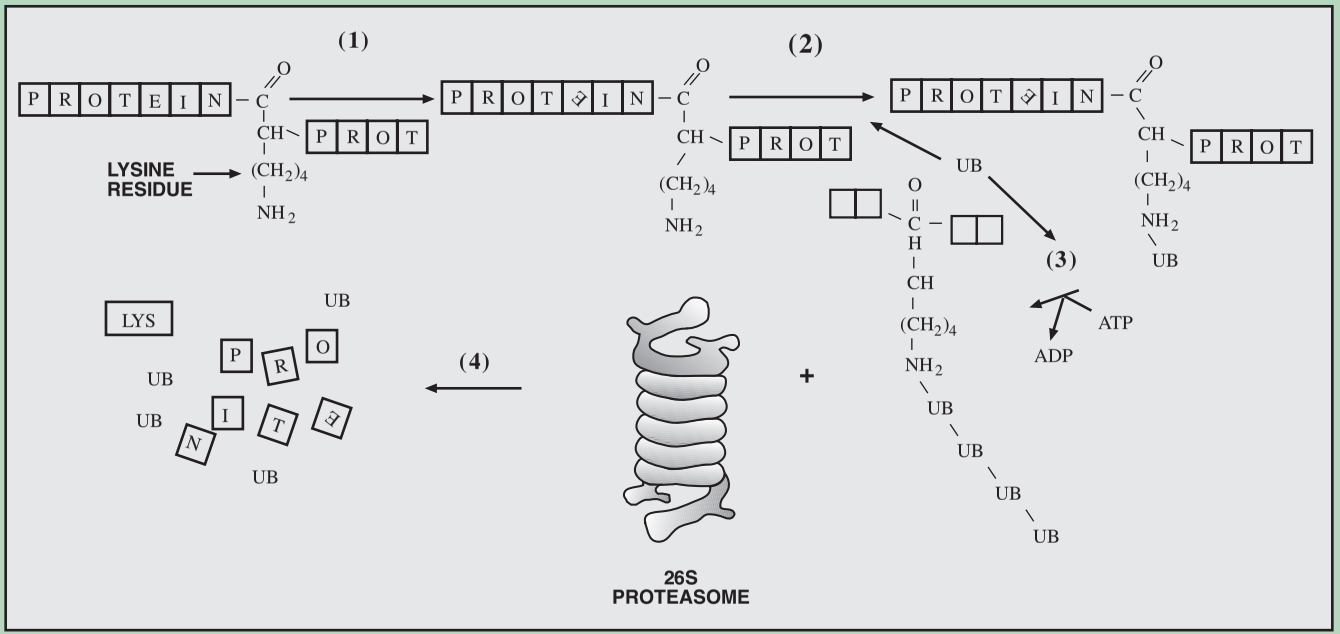
Cataract formation usually starts with oxidative damage to the fiber cells of the lens. Environmental exposures to UV light and to smoking increase the rate of oxidative damage, which is reflected in increased risk for cataracts. Oxidative damage causes the long chains of lens proteins to become crosslinked with sulfur-sulfur bonds between sulfhydryl groups on adjacent protein chains. When there is enough glutathione (a potent antioxidant), these crosslinks can be reversed. The number of abnormally modified and aggregated lens proteins also increases through chemical reactions between the proteins and either carbohydrates or peroxidation products of PUFAs. The relative amount of these abnormal proteins (e.g., glycation products) increases with age, another indicator of the cumulative effect of oxidative damage. In general, these modifica-

tions cannot be reversed. The mechanism for increased oxidative damage from smoking, Dr. Taylor suggested, is that smoking or smoke-related byproducts (1) damage the bulk proteins of the lens or other tissues, and (2) deplete the body's stores of vitamin C or other antioxidant nutrients. Vitamin C (in the aqueous fluid of the eye) helps protect the lens from oxidative damage.

Specific enzymes that break down damaged proteins into their constituent amino acids (proteolytic enzymes) are one of the mechanisms for removing products of oxidative stress. The entire process of identifying damaged proteins, marking them for degradation, and degrading them into amino acid constituents forms a healthy cell's *protein-editing machinery*. Dr. Taylor described work done by his group on ubiquitin, a protein that binds to other proteins to mark them for degradation by protease enzymes (figure 6) [22, 23, 24]. When heat or oxidative damage causes a protein to unfold from its normal functional configuration, it exposes segments of the protein (called degrons) that bind to ubiquitin to form the starting point for protease degradation. In young lenses, this ubiquitin-marking pathway functions well in getting damaged proteins removed. However, in older lenses, the ubiquitin-conjugated proteins tend to accumulate, rather than being removed by the protein-editing machinery. The ubiquitin conjugate is typically much larger than the original damaged protein, and if that conjugate gets linked to another damaged protein, the whole protein mass is large enough to precipitate from solution as an opaque deposit: the biochemical definition of cataract formation. In short, Dr. Taylor posits that, if the protein-editing machinery cannot keep up with the rate of formation of damaged proteins, the ubiquitin-binding step in the normal ubiquitin pathway can actually contribute to cataract formation. This slowing of the protein-editing machinery probably occurs in some form in many tissues of the aging body, Dr. Taylor said, but the consequences are manifested differently depending on how the healthy functions of the tissue are altered.

When lens cells are challenged with increased concentrations of sugars or sugar derivatives, the degradation of abnormally modified crystallin proteins slows down. The protein-editing machinery is compromised. If the protease enzymes, which are themselves proteins, are being

Figure 6. The Ubiquitin Pathway for Removing Damaged Proteins



Note: (1) A protein sustains oxidative damage (indicated by the distorted "E" in the protein sequence). (2) A first ubiquitin molecule is attached to a lysine residue in the damaged protein. (3) Three or more additional ubiquitin molecules are conjugated in a chain on the damaged protein. (4) The ubiquitin-protein conjugate is broken down into its constituent amino acids and ubiquitin molecules by a 26S proteosome.

damaged, then the removal of other proteins damaged by oxidative stress is hindered. The accumulation of damaged proteins increases precipitation of high-mass conjugates, hastening the formation of cataracts. Over several years of work, Dr. Taylor's group has shown that the presence of antioxidants and maintenance of a low concentration of simple carbohydrates (sugars and sugar derivatives) can slow the inactivation of proteases in the protein-editing machinery and therefore slow or prevent the harmful accumulation of abnormal ubiquitin conjugates.

Epidemiologic Data on Dietary Antioxidants and Carbohydrates

These results lead to the question of whether managing patterns of nutrition can benefit the biochemical environment of the lens, protecting and enhancing the natural protein-editing machinery while slowing the rate of oxidative damage to both crystallin proteins and proteases in the fiber cells. Dr. Taylor described research results that shed light on two important questions: (1) Do nutrients in the diet or from dietary supplements get to the lens (and/or to the retina, where oxidative damage and protein-editing pathways also appear to be critical to healthy vision)? (2) Is there any relationship between disease state and concentration of antioxidants in these eye tissues? Regarding the first question, there seems to be a correlation between dietary intake and levels of some nutrients. Although intervention trials have not conclusively shown a protective effect for antioxidants, associations found in observational studies suggest there may be an effect. For AMD, the AREDS intervention trial did suggest that progress to advanced AMD could be delayed in at-risk individuals. This finding should be corroborated. On the second question, observational studies by a number of groups have found no consistent relationships between lens disease prevalence and dietary intake or blood levels of a number of antioxidants and minerals, including ascorbate, glutathione, tocopherols, lutein/zeaxanthin, retinol esters, zinc, copper, and calcium [25]. However, actual tissue levels of these nutrients have typically not been determined and so have not been correlated with dietary intake or blood levels.

To determine whether there was an association between carbohydrate intake and increased risk of cataract, Dr. Taylor's group has ana-

lyzed epidemiologic data from several cohort studies that collected nutrition information from food questionnaires and also graded subjects' eyes for retinal lesions and for opacity of the lens cortex and nucleus (core). The analyses of nutrition included estimates of the subjects' quantity of carbohydrate intake and the quality of this intake (simple sugars, or those more readily metabolized to simple sugars, versus complex carbohydrates, which are more slowly metabolized). In the Nutrition and Vision Project cohort within the larger Women's Health Study, the analysis found about twice the risk of cataract for the group with highest carbohydrate intake compared with lowest carbohydrate intake (highest versus lowest third) [26]. For the AREDS cohort, there was increased risk for both higher levels of carbohydrate intake and lower carbohydrate quality (as measured by a glycemic index that correlates with the glucose serum level produced by a food in the first two hours after eating) [27]. Analyses for two other cohorts have also shown increased risk of cataract with increased carbohydrate intake. When a similar analysis was done for carbohydrate intake and AMD in the AREDS cohort, the evidence for an increased risk was even stronger than for carbohydrate intake and cataract. Not only was risk for all grades of AMD decreased but also progress of AMD was slowed in persons who consumed diets with a lower dietary glycemic index. Dr. Taylor noted that the difference between being in the high versus low groups with respect to this dietary glycemic index can depend on as small a dietary change as consuming five slices of whole wheat bread, rather than five slices of white bread [28].

Post-Presentation Discussion

After Dr. Taylor's presentation, he discussed with Dr. West, Dr. Emily Chew, and Dr. Chader whether a clinical relationship has been established between lens brunescence (brown coloration) and visual impairment, including increased risk of developing evident opacity and cataract. There was general agreement that the Lens Opacities Classification System III (LOCS) separated brunescence from opacity or opalescence [29], and most of the participants interpreted the work of the LOCS Study Group as finding no association between brunescence and the

progressive opacity or cloudiness that leads to cataract and visual impairment. Dr. Taylor suggested that, because brunescence is induced after glycation, a better understanding of the underlying biochemical mechanisms and pathways would contribute to understanding and treating both pathological brunescence and opacification of the lens, even if they are distinct processes.

Dr. Nicolas Bazán commented that the presentation had brought together concepts that are likely to be important in the development of both cataract and AMD. Protein misfolding may be a major event in initiating disease mechanisms in these and other chronic diseases. For example, polyglutamine-containing proteins that are misfolded are linked to some forms of retinal degeneration. He and Dr. Taylor discussed potentially important features of enzymes in the ubiquitin pathway whose activity is affected by nutrient signaling, including signaling by antioxidant nutrients. One class of such enzymes, called chaperones, normally aids a protein in folding into its functional configuration. Dr. Taylor described work by his research group in exploring how different components of the protein-editing machinery, such as these chaperone proteins, can be turned off and on with nutrient signaling.

Dr. Caroline Klaver asked if antioxidant supplements might help prevent pediatric cataract tied to defects in the genes for crystallin proteins. Dr. Taylor said he did not know of any evidence that antioxidants would help in that situation. Dr. Chew, Dr. Klaver, and Dr. Taylor discussed whether the associations of carbohydrate intake and glycemic index with cataract were supported by an association of higher cataract or AMD risk for patients with diabetes. Dr. Chew said that AREDS had not found an association of AMD with diabetes, and Dr. Klaver said the same for the Rotterdam Eye Study. Dr. Taylor said that he believes there is significant commonality of biochemistry across many different tissues—for example, the ubiquitin pathway for removing damaged proteins occurs in many other tissues of the eye as well as throughout the body—but that the triggering events and pathways related to a tissue-specific disease such as AMD or cataract may differ because of the functional differences from tissue to tissue. Finding and understanding this commonality or "crosstalk" among disease pathways will require more

work, he suggested, but if successful it should improve our understanding of the subtleties in the environments and pathways that lead to disease endpoints and potentially yield better treatments applicable to more types of tissue.

Dr. West asked for Dr. Taylor's thoughts on why, given the general understanding of the protective role of vitamin C as an antioxidant in aqueous environments, the large doses of vitamin C used as a dietary supplement in AREDS had not produced a beneficial effect. Dr. Taylor agreed that it is difficult to reconcile the results from AREDS and other trials of vitamin C supplements with the presumed role of antioxidants in protecting cellular functions from oxidative damage. He noted that Americans are well nourished with respect to getting the minimum daily requirement of essential nutrients such as vitamin C. Even in the rest of the world, diets are not often severely deficient in vitamin C. Some of his data show that, beyond a certain ceiling level, higher vitamin C concentration in a tissue does not have a measurable beneficial effect. He and the other participants discussed whether recent clinical trials involving vitamin C supplementation would have involved conditions where a signal of treatment efficacy would be detectable and statistically significant. A recurring thought was that having enough of a vitamin in the diet may suffice; increasing dietary intake may have little or no beneficial effect (and may at some elevated level of intake even produce negative effects).

Dr. Hetherington asked about ways of looking for connections of glycemic effects on lens tissue with the genetics of producing and maintaining insulin in the body. Dr. Taylor replied that, in cooperation with the AREDS investigators, his group is studying the insulin signaling pathway in lens tissue to determine whether there is a genetic susceptibility to lens protein damage from increased glucose concentration (higher glycemic index). Dr. Chader asked about biomolecules in addition to glutathione that regulate the ubiquitin pathway. Dr. Taylor described work showing that the ubiquitin protein-editing pathway can be protected or restored to normal functioning by adding antioxidants when the cellular environment is challenged by addition of peroxide at levels that can occur in the aqueous humor (i.e., physiologically meaningful concentra-

tions). Dr. Bazán supported Dr. Taylor's view that control and modulation of the ubiquitin pathway is immensely important for understanding disease pathways where protein damage is or may be a key factor.

Nutrition, Environment, and Diabetic Retinopathy

Dr. Rohit Varma

Diabetic retinopathy (DR) is one of the leading causes of new cases of legal blindness among working-age Americans. The number of new cases each year works out to another American blinded by DR every 20 minutes. About a third of those who have DR are unaware that they have it. DR is a long-term consequence of diabetes—a chronic and often progressive disease in its own right. The growing number of Americans diagnosed with type 2 diabetes (figure 2, p. 27) means that DR will continue increasing as a cause of blindness for many years, unless ways are found to halt or slow the initiation and progression of this consequence of a systemic imbalance in blood sugar level.

With respect to causal factors for DR, biological risk factors are proximate causes, but dietary and environmental factors act strongly in the background. They have impacts on the biological factors directly related to prevalence and incidence. Exactly how the principal biological risk factors—duration and severity of high levels of blood sugar (hyperglycemia)—cause DR to start and progress is not known. Dr. Varma reviewed the evidence for parts of the disease pathway. He described DR as essentially a disease of the small blood vessels in the retina. Degeneration of the pericytes—the cells of the connective tissue that surrounds the endothelial cells forming the vessel wall—is the first pathological lesion seen in DR. As the disease condition continues, these pericytes undergo programmed cell death (apoptosis), and the loss of pericytes leads to weakening of the blood vessel walls and formation of micro-aneurysms (small, balloon-like projections from the wall). Thickening of the basement membrane occurs, which may lead to occlusion (obstruction or shutting off) of the capillaries. Progressive capillary

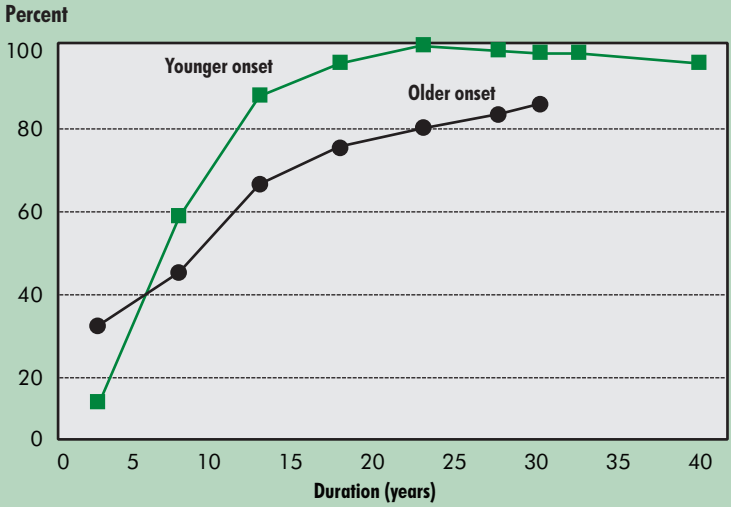
occlusion blocks blood flow in the retinal capillaries (ischemia), which in turn may stimulate new blood vessel formation (neovascularization) through increased production (up-regulation) of vascular endothelial growth factor (VEGF).

Leakage from ruptured capillaries into the extracellular space of the retina results in diabetic macular edema, which threatens central vision. The advanced disease stage in which new but fragile blood vessels are forming and themselves leaking is proliferative diabetic retinopathy (PDR). Outcomes from PDR can include substantial hemorrhages on the inner retina and retinal detachment. The stages prior to VEGF-mediated proliferation of new blood vessels, when there is mild to severe capillary damage and swelling of the retina from leakage, are referred to as "nonproliferative diabetic retinopathy."

The biochemical changes that trigger pericyte apoptosis are not yet conclusively established; they may involve accumulation of toxic metabolic products such as sorbitol or advanced glycation end-products (AGEs). AGEs can be produced by inappropriate binding of glucose to protein side chains, so this may be the link between hyperglycemia and the initiation and promotion of DR [30]. Bioactive molecules, such as aminoguanidine and pimagedine, that inhibit either AGE formation or the binding of AGE to receptors have been tested for efficacy in animal models of DR. A randomized clinical trial of pimagedine is in progress [31].

Based on epidemiologic studies, the duration of time that a person has diabetes is a major risk factor for developing DR (figure 7). Four out of five individuals who have had diabetes for at least 15 years have some stage of DR. This statistic applies to both the early-onset (younger than 30 years old) type 1 diabetes, which has a major genetic component, and the later-onset, type 2 disease. The prevalence of type 2 diabetes has been increasing in conjunction with the American trend toward over-eating and obesity. Another well-established risk factor for DR is the severity of the subject's hyperglycemia—how far a diabetic's blood sugar ranges above normal levels. Several landmark clinical trials have shown that effective control of blood glucose levels to near-normal levels can slow DR incidence and progression in diabetic individuals.

Figure 7. Prevalence of Any Retinopathy by Duration of Diabetes



Source: Wisconsin Epidemiologic Study of Diabetic Retinopathy, 1980–82; data courtesy of Ronald Klein, M.D., M.P.H.

Given the importance of these two risk factors, early diagnosis and treatment of DR is crucial for both the quality of life of the patient and for public health policy. Early detection of DR and treatment of the underlying diabetes with robust control of blood sugar can delay disease progression and is cost-effective compared with untreated outcomes [32–39]. Therefore, once an individual has been diagnosed with diabetes of either type, annual eye examinations to watch for signs of DR are recommended. An unfortunate environmental-cultural factor that undermines this treatment strategy, Dr. Varma emphasized, is that the general medical practitioners or internal medicine physicians who routinely see patients with diabetes often do not follow through on ensuring their patients get annual eye examinations. Close coordination between primary care physicians and ophthalmologists to catch DR at its earliest phase is both a reasonable standard of care and sound public health policy.

High blood pressure is another risk factor for incidence and progression of DR in type 2 diabetes. In the United Kingdom Prospective

Diabetes Study, tighter control of blood pressure toward normal levels reduced the risk of DR [37]. Subsequently, the Appropriate Blood Pressure Control in Diabetes trial did not find a further decrease in risk if the blood pressure range in hypertensive type 2 diabetics was controlled even more tightly than in the U.K. study [40]. However, a follow-up to this trial by the same researchers showed that, for type 2 diabetics who started with blood pressure in the normal range (<140/90 mm Hg), maintaining their diastolic pressure at or below 75 mm Hg reduced the risk of DR progression relative to maintaining diastolic pressure in the range of 80–89 mm Hg. In this follow-up study of diabetic subjects with normal-range blood pressure, the group with more intensive control of blood pressure also had less risk of progression to incipient and overt diabetic nephropathy (kidney disease) and decreased the incidence of stroke [41]. For type 1 diabetics with normal-range blood pressure, treatment with lisinopril, an antihypertensive medication that inhibits angiotensin-converting enzyme, slowed the progression of both diabetic nephropathy and DR [42].

Other risk factors for incidence and progression of DR that may have an environmental and/or nutritional link include elevated serum cholesterol and triglycerides [43] and ethnocultural identity. With respect to the latter, Latino Americans have a 6- to 8-fold higher prevalence of DR compared with Americans of non-Latino European ancestry, even after accounting for differences in blood glucose control and blood pressure control [44]. African Americans and Americans of non-Latino European ancestry had similar rates of DR progression once differences in blood glucose control and blood pressure control were taken into account[2] [45].

Dr. Varma presented two severity scales currently used to assess the stage of DR in patients. He explained why early screening and proper assessment of disease stage is important for progression prognosis and

[2]As emphasized in the report from the Fourth Drabkin Symposium, the conventional demographic categories for special populations such as White, Hispanic or Latino American, and Black (or African American) are poor markers of genetic differentiation. Differences in disease risk associated with these ethnocultural identities may be due to environmental-cultural differences, including dietary patterns, as well as to differing genetic susceptibilities [4, pp. 11–15, 49–55].

disease management. Disease management entails managing all the disease stages that require treatment, with timely intervention when a treatable disease condition first appears. Treatment can be systemic therapy, nonspecific local therapy, or a combination. The overall goal is to prevent vision loss or at least delay it as long as feasible. The proximate causes of vision loss are macular ischemia (loss of blood flow to the macula), clinically significant macular edema, and the consequences of neovascularization in PDR. A major goal of prognosis is to go beyond diagnosis of the current disease stage and predict the risk and probable timing of progression to the next stage.

Dr. Varma reviewed both proven and experimental systemic therapies and nonspecific local therapies, summarizing the benefits and potential risks (e.g., toxicities and side effects) of each. The nonspecific therapies include several types of photocoagulation for PDR, in which a laser beam is used to cauterize parts of retinal tissue to interrupt the progression from neovascularization to capillary leakage, blood vessel hemorrhaging, and scarring of the retina. Vitrectomy, which is another nonspecific local therapy, is used to try to reduce severe vision loss in patients with nonclearing vitreous hemorrhage or other consequences of advanced PDR. There are several experimental therapies for macular edema, including vitrectomy.

From the perspective of environmental factors in DR disease management, Dr. Varma stressed some major limitations in current medical interventions and how they can be improved.

- Timely detection and treatment of DR could prevent many new cases of diabetic blindness, but screening rates are extremely low in some populations. For example, in some communities of Los Angeles with a high proportion of Latino Americans—a special population with elevated risk of DR—less than 2 percent of the community has been screened. The reasons why screening rates are so low include barriers to access the necessary care, lack of education and awareness, and the fact that DR is frequently asymptomatic until it reaches an advanced stage.
- The current techniques for intensive systemic care of the underlying diabetes, such as tight control of blood sugar to prevent

hyperglycemia and tight control of blood pressure to stay in the low-normal range, have their own toxic consequences. Better methods are needed to more closely mimic the biochemistry of normal insulin and blood glucose management.

- The current local therapies, including photocoagulation techniques, are nonspecific and destructive of retinal tissue and function. Because these treatments do not target the underlying pathogenesis, e.g., pericyte loss, they do not address some mechanisms of vision loss from the underlying condition, such as macular ischemia. Given their significant toxicity, these therapies are not advisable for early or preventive treatment.

In response to questions from symposium participants about repetitive scatter panretinal photocoagulation, Dr. Varma said the factors in a decision to repeat this laser technique include not only the condition of the retina but also the patient's blood sugar management. If tight glycemic control prevents high glucose levels in the retinal small blood vessels, then the ischemia that induces the neovascularization response can be avoided and retreatment may not be needed. In the laser treatment, small burn spots are scattered across the retina (except for the macula) to avoid photocoagulating larger blood vessels, which could in itself induce ischemia and neovascularization.

As mentioned above, by far the most important environmental factor in America that is driving the increase in prevalence of diabetes—and therefore the prevalence of DR and other complications of hyperglycemia—is the cultural trend toward obesity. In many developed countries, being overweight is due to overeating and to dietary patterns that are inappropriate for modern lifestyles. Changing the diet, limiting total caloric intake and particularly certain kinds of foods, and increasing regular exercise decreases the risk of hyperglycemia and type 2 diabetes. These same lifestyle changes are also vital to managing type 2 diabetes after it develops.

Effective screening programs that reach a much larger proportion of the population with hyperglycemia, in every ethnocultural community and at every socioeconomic level, are essential to the timely detection and treatment of DR. Dr. Varma described recent successes with

screening programs that incorporate telemedicine technology to bring expert care to where the patients are, instead of trying to get patients to the ophthalmologist. Testing sites equipped with nonmadriatic cameras (cameras that do not cause pupil dilation) have been located in shopping malls and other community locations to capture fundus photographs, which are transmitted to centralized reading centers for evaluation. The Joslin Vision Network and the Inoveon Diabetic Retinopathy System are two programs in which this approach has been successful. The evaluation only needs to determine which individuals need care now and which do not. A further step in automation is now feasible by using analysis software to identify DR lesions accurately and objectively.

After effective screening programs, better systemic control of insulin and blood glucose levels is the next essential step in managing care of the diabetic patient to prevent DR incidence and progression. Experimental approaches to improve systemic control include surgical procedures to restore natural insulin secretion, such as pancreas transplants or transplants of portions of the pancreas (solitary islets of Langerhans, which secrete insulin). Among new pharmacologic approaches are insulin analogues that are designed for sustained release from a subcutaneous injection. Pharmacologic approaches to specific local therapy include therapeutic agents that intervene in the formation of AGEs or otherwise protect pericytes from oxidative damage, as well as anti-VEGF agents aimed at preventing the proliferation of new blood vessels. Several clinical trials of candidate therapeutic agents are under way. For all these potential advances in treating and managing DR, an invaluable part of the infrastructure to support clinical studies is the Diabetic Retinopathy Clinical Research Network, a collaborative network dedicated to facilitating multicenter clinical research on DR, diabetic macular edema, and associated conditions.

Post-Presentation Discussion

On the potential role of nutrition in managing DR, Dr. Emily Chew asked about scientific evidence supporting claims that have been made for vitamin E as an antioxidant in controlling DR. Dr. Varma replied that he had not seen strong evidence supporting a beneficial effect, although

some small studies reported finding a benefit. Dr. Nicolas Bazán asked if Dr. Varma had any thoughts on the higher DR risk for Latino Americans compared with African Americans, given that the latter have an elevated risk, relative to the general population, for cardiovascular disease. Dr. Varma replied that, even after controlling the epidemiologic data for blood glucose control, blood pressure control, access to care, and other variables, Latino Americans still had an elevated risk for DR, so some underlying predisposition, genetic or otherwise, is a possibility. A detailed assessment of nutrition and diet for these two special populations, in relation to DR prevalence, has not yet been done. The participants agreed that one dietary factor worthy of further study would be differences in fish consumption (and other sources of PUFAs) between these two groups. Dr. Sheila West noted that there has not yet been a major epidemiologic study that has directly compared African Americans, Latino Americans, and Americans of non-Latino European ancestry. An issue with comparisons across separate studies is that the definitions of degree of glycemic control are not the same, which could skew the comparison.

Nutrition, Environment, and AMD

Dr. Caroline Klaver

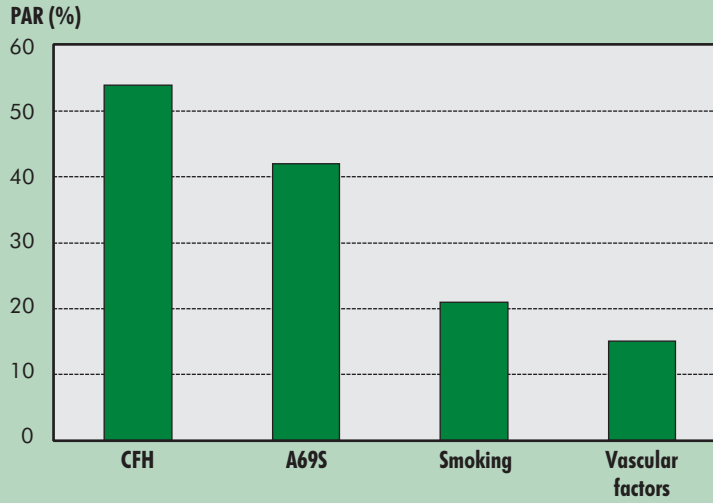
Dr. Klaver acknowledged the support of her colleagues, Dr. Redmer van Leeuven and Dr. Hans Vingerling, in preparing the presentation for the symposium, as well as Dr. Paulus de Jong, principal investigator for the ophthalmology portion of the Rotterdam Study, from which much of the data in the presentation derives. With respect to prevalence of AMD, epidemiologic studies of various populations around the world suggest that prevalence rates for both early-stage AMD (soft, distinct drusen with or without pigmentary changes) and late-stage AMD (neovascularization or geographic atrophy) are higher in populations of primarily European ancestry (for example, the Beaver Dam Eye Study in Wisconsin, the Rotterdam and EUREYE studies in Europe) than in populations with a large fraction of African ancestry. Populations of mostly Asian

descent have prevalence rates of early AMD closer to European-ancestry populations, but their late-stage AMD rates are much lower. These and other results, such as twin and family aggregation studies, lend support to the generally accepted view that genetic disposition plays a substantial role in risk for developing AMD.

During the past decade and more, the relative importance for AMD pathogenesis of oxidative damage and inflammatory processes has been a topic of debate in the research community. Beginning in 2005, however, results from studies of polymorphisms (naturally occurring variants) in several genes associated with increased AMD risk have provided evidence that local inflammatory processes in the retina are more important than oxidative damage, although oxidative damage remains important. The Y402H variant of the gene for complement factor H (CFH) occurs more frequently in AMD patients than in age-matched controls [46, 47, 48]. CFH is an inhibitor of the complement cascade that occurs as part of an inflammation response. The Y402H variant appears to decrease CFH's binding to C-reactive protein. Since normal binding between these molecules increases the inhibitory effect of CFH, decreased binding may lead to overactivation of the complement cascade. An analysis of the association between this gene variant and AMD in the Rotterdam Eye Study population found that the population-attributable risk (PAR) of AMD for this CFH variant is 54 percent [49]. The A69S variant of the LOC gene, which has been shown to be located in the mitochondrial outer membrane, appears to interact with smoking to increase the risk of AMD independent of the CFH/Y402H variant [50]. Figure 8 presents the analysis by Dr. Klaver's group of the PAR for these three AMD risk factors, plus atherosclerosis/hypertension, as they appear in the Rotterdam Eye Study population.

Despite the substantial role that genetic susceptibility apparently plays in AMD risk, environmental factors (such as smoking) and diet play an important role that can affect AMD risk for those with a genetic susceptibility. As part of the Rotterdam study, a prospective analysis was performed on dietary data for subjects aged 55 and older at the beginning of the study (1990–93 baseline). The group with incidence of early-stage AMD at any time during three rounds of follow-up (last

Figure 8. Population-Attributable Risk for Four AMD Risk Factors in the Rotterdam Eye Study



Note: CFH is the Y402H variant of the gene for complement factor H. A69S is a variant of the LOC gene. Vascular factors include atherosclerosis and hypertension. PAR represents the proportion of the disease (here, late AMD) that can be attributed to the specified risk factor.

follow-up in 2000–04) was compared with subjects who had not developed early-stage AMD during this period. Dietary data were gathered at baseline and in the follow-ups using a semiquantitative food intake frequency questionnaire. Of the 4,170 study participants, 518 developed early-stage AMD and 42 progressed to late-stage AMD. The nutrients studied included carotenoids (alpha- and beta-carotene and lutein/zeaxanthin[3]); lycopene; vitamins A, C, and E; and the bioactive minerals iron and zinc. A univariate analysis was performed on level of nutrient

[3]Lutein and zeaxanthin are chemical isomers: they have the same chemical composition and differ only in the location of one carbon-carbon double bond. Because they are chemically very similar and difficult to distinguish by basic nutrient analysis techniques, the concentration of their combined amount is typically reported. Lutein and zeaxanthin are concentrated in the retina, particularly in the macula, where they are thought to act as antioxidants.

intake by quartiles (i.e., the study population was divided into four groups by amount of the nutrient eaten). For the carotenoids, which are strong antioxidants, this analysis showed no statistically significant reduction in risk for even the highest quartiles, relative to the group eating the lowest amount of the nutrient (lowest quartile) [51]. As a caveat on drawing conclusions from these results, Dr. Klaver noted that this study population does not eat large amounts of the kinds of vegetables that are good dietary sources of these carotenoids. The estimated daily intakes of these nutrients were low relative to the amounts recommended by nutrition experts [51].

Similarly, the data from the Beaver Dam Eye Study and from the Health Professionals study of nurses and physicians show no significant dose-response relationships when analyzed in a similar manner for dietary beta-carotene and lutein/zeaxanthin. However, as in the Rotterdam study, the estimated daily intakes are low relative to recommended levels. The Health Professionals study population was predominantly later-stage AMD patients, whereas the Beaver Dam population, like that in Rotterdam, was primarily early-stage AMD or no AMD [52, 53].

The dietary intake of vitamin C by the Rotterdam study population includes sufficient amounts of fruit to have the lowest quartile of vitamin C intake at 70 percent of the recommended daily allowance (RDA) and the highest quartile at twice the RDA. However, the univariate analysis comparing AMD risk for the higher quartiles against the lowest quartile showed no significant protective effect by vitamin C. In the Beaver Dam and Health Professionals studies, the point estimates (mean value) for the odds ratio of risk in the higher quartiles relative to the lowest quartile suggested some protective effect, but the uncertainties (95 percent confidence intervals) in the estimates were large enough that the results are not statistically significant.

For vitamin E, the Rotterdam study found a statistically significant protective effect for the highest quartile of intake (about 1.3 times the RDA) compared with the lowest quartile (half the RDA). A similar result appears in the Beaver Dam population, with large drusen (early-stage AMD) as the predominant form. For the Health Professionals study population with predominantly later-stage AMD, there was no protective effect. The highest quartile in the Health Professionals study

was estimated to be getting only about 80 percent of the RDA, with the lowest quartile at about the same level as in the Rotterdam study. The symposium participants discussed several methodological differences between the Health Professionals study and the Beaver Dam and Rotterdam studies that may account for differences in the estimates of relative AMD risk. These study differences include disease endpoint and verification of disease stage, differences in food intake questionnaires and method for calculating daily dose of nutrient from questionnaire data, and differences in subjects' overall response to completing the questionnaires (response bias).

Zinc is an essential cofactor for many of the antioxidant enzymes in the body. The normal concentration of zinc in the retina is higher than in other parts of the body. Food sources of zinc are common in the Dutch diet, and the Rotterdam study population had 85 percent of the RDA in the lowest quartile of zinc dietary intake, with 1.4 times the RDA in the highest quartile. There appeared to be a slight protective effect, Dr. Klaver said, even at the second-lowest quartile (odds ratio of 0.86 relative to the lowest quartile, 95 percent confidence limits of 0.68 and 1.10), with a dose-response relationship in the point estimates for the three higher quartiles. The Beaver Dam results are comparable. (No data on zinc were available from the Health Professionals study.)

The data for the Rotterdam study group were also analyzed for the combined effect on AMD risk of the antioxidant nutrients that had shown the most benefit in slowing late-stage AMD progression in the AREDS trial of dietary supplements (table 3). The four nutrients involved in the analysis were vitamins C and E, beta-carotene, and zinc. The reference group for this analysis included all the Rotterdam study subjects whose dietary intake for each of these nutrients was in an "average" range (3,270 of the 4,170 participants, or 78 percent) between the high and low extremes. At either end were a much smaller group with the lowest dietary intake of the four nutrients (466 participants) and a group with the highest dietary intake (434 participants). The upper portion of table 3 shows the estimated dietary intake of each of the four nutrients in each of these three groups: low intake, mean intake, and high intake. The hazard ratio for each group is its risk of AMD rela-

tive to the mean-intake group, after adjusting for a range of factors that may influence disease progression such as age, smoking, cardiovascular health, gender, and others (see complete list in the note to table 3). Using this analytical approach, the low-intake group had a 20 percent higher risk of AMD than the mean-intake group, whereas the high-intake group had 35 percent less risk of AMD than the middle group. The right-hand column of table 3 indicates where the high-intake group falls with respect to the RDA for each of the four nutrients. To illustrate what these results imply about the importance of a healthy diet for ameliorating the personal losses and public health costs of AMD—despite the significant genetic component in this disease—the Rotterdam researchers drew the following hypothetical scenarios based on the combined nutrient analysis:

- Assume that the entire population of the Netherlands age 55 and older with no present signs of AMD (about 3.8 million people) currently eats a diet that falls in the average-intake group.

Table 3. Combined Dietary Intake of AREDS Ingredients in Rotterdam Eye Study Population

	Low (n = 466)	Mean (n = 3,270)	High (n = 434)	High versus RDA
AREDS ingredient (mg/day)				
Vitamin C	77	116	150	2×
Vitamin E	9	13	16	~1×
Beta-carotene	3	4	5	~1×
Zinc	9	11	12	1. 4×
Hazard ratio	1. 20 (0.92–1.56)	1. 00	0. 65 (0.46–0.92)	

Note: Hazard ratio adjusted for age, sex, body mass index, smoking, systolic blood pressure, atherosclerosis, serum cholesterol, and alcohol intake.

Source: Rotterdam Eye Study; data courtesy of Dr. Caroline Klaver.

- After 10 years, one would expect, based on the Rotterdam data, that 287,422 of these people would have early-stage AMD and 28,742 would have developed late-stage AMD.
- If this entire population shifted now to a diet that provided the daily intake levels characteristic of the high-intake group, the Rotterdam Study data imply that, after 10 years, the incidence of AMD would be 35 percent less. In other words, there would be 100,598 fewer cases of early-stage AMD and 10,060 fewer cases of late-stage AMD than if the current "average diet" were followed.

Dr. Julie Mares has estimated that, if the hazard ratios calculated from the Rotterdam data hold for the American population as well, and assuming the mean-intake diet is the typical diet for Americans 55 or older with no current signs of AMD, then switching that entire population at risk of AMD to the diet of the Rotterdam high-intake group would be expected to prevent 461,000 cases of advanced AMD in five years [54]. These scenarios illustrate the potential value of a diet rich in antioxidants and zinc in slowing the expected growth in vision loss from late-stage AMD.

The symposium participants discussed some of the assumptions underlying these hypothetical scenarios, such as the proportion of late-stage AMD that would be prevented for a given reduction in early-stage AMD incidence. In response to a question from Dr. Sheila West, Dr. Klaver said that a project she is undertaking will be a multivariate analysis for combinations of risk factors, such as the AMD-associated gene variants and the known environmental factors such as smoking and diet. In response to a question from Dr. Rohit Varma about potential confounders in assigning risk ratios to dietary intake characteristics, Dr. Klaver agreed that the results of a single study are highly subject to all the assumptions made in the epidemiologic analysis. An important consideration is the analytical approach used to adjust the raw observational data for both established and potential risk factors that could be confounders. One population study does not prove anything, but it does suggest next steps worth taking. She agreed that the lack of data about intermediate concentrations (i.e., serum and retinal tissue concentra-

tions) of the bioactive species from antioxidant uptake and metabolism are important for establishing a causal explanation of the role these nutrients may play in slowing disease progression. Experimental data that trace and confirm the pathways to disease stages will be necessary for that level of explanation. The participants also discussed the current information on the relation of dietary intake of lutein/zeaxanthin to concentrations of their metabolites in serum and the retina.

With respect to associations between dietary fats, including PUFAs, and AMD risk, Dr. Klaver said there were no analyses yet from the Rotterdam study. She did summarize the evidence from other research reported in the literature. Most studies report some degree of decreased AMD risk for diets containing more fish, and the level of omega-3 PUFAs in these lower risk diets appears to be a consistent factor across multiple studies [55].

Smoking is the environmental risk factor for AMD with the longest and best established track record for definitive association. As far back as 1978, the association of smoking with earlier incidence of AMD was documented in 114 AMD patients. The average age of 64 years at onset for smokers contrasted with an average age at onset of 71 for AMD patients who had never smoked. The authors advised all patients with signs of AMD to stop smoking [56]. Studies since then have consistently confirmed the association of smoking with increased risk for AMD. Nearly 30 years later, the EUREYE study of almost 5,000 subjects aged 65 years or older in seven European countries found that current smokers have an increased risk for both geographic atrophy and neovascularization (odds ratio of 4.81 for geography atrophy, 2.56 for neovascularization, compared with those who never smoked). Furthermore, stopping smoking decreases the risk for both forms of late-stage AMD [57]. Additional confirmation of smoking as a risk factor has come from the Health Professionals studies, Blue Mountain Study, and the Rotterdam Study. Potential mechanisms by which smoking may promote AMD progression are numerous. The tar in tobacco smoke contains numerous compounds that promote oxidation, including many chemicals with a quinone structure, such as hydroquinone. Hydroquinone produces free radicals and promotes production of superoxide, compounds that mediate protein

oxidation and lipid peroxidation. Thus, smoking may directly promote AMD pathogenesis through formation of free radicals, peroxidation of lipids, and disruption of normal functioning by the RPE or by indirect effects such as atherosclerosis of the choroid behind the retina.

As her last topic, Dr. Klaver returned to the genetic component of AMD risk. She presented data on the epidemiologic risk of late-stage AMD for combinations of six gene variants, each of which is associated with either an increased risk (odds ratio greater than 1) or decreased risk (odds ratio less than 1) of late-stage AMD. Two of these are variants of the CFH gene, with the Y402H variant being a risk factor for AMD and the intron14 variant being protective for late-stage AMD (odds ratio less than 1). The other four variants are each in a different gene. Of these six variants (single-gene polymorphisms), three increase the risk for late-stage AMD; three decrease it. Dr. Klaver reviewed the current knowledge about the role of the protein expressed by each gene and how the variants of the gene may affect AMD pathogenesis. Based on literature results and unpublished data from the Rotterdam study, Dr. Klaver summarized the risk (odds ratio) for late-stage AMD of having one or two alleles of each of these variants, relative to the risk with none of the six variants. A case-control study by other authors, published in 2006, calculated a "best case/worst case" odds ratio of 250 for persons who are homozygous (two alleles) for all three increased-risk gene variants and for none of the protective variants, relative to persons who are homozygous for all three protective variants and none of the risk-increasing variants [58]. Using the same underlying genetic data from about 2,000 subjects with late-stage AMD and 1,000 with no AMD signs, Dr. Klaver's group defined the reference risk for comparison as the risk of late-stage AMD in the general population, aged 65 or older, which is about a 3 percent risk (i.e., prevalence of late-stage AMD is about 3 percent). Relative to this reference class, the risk for the worst-case gene variant combination is 14-fold greater (odds ratio of 14); the best-case combination has a 20-fold lower risk of late-stage AMD (odds ratio of 0.05).

In response to a question about how smoking affects the AMD risk with these genetic factors, Dr. Klaver said that the case-control data did not allow for that analysis, but she is currently investigating the subject

for the Rotterdam study population. The medically interesting part of this research area, she said, is that environmental factors do appear to influence the impact of these genetic factors on disease incidence in at-risk individuals. For example, the risk of late-stage AMD for current smokers who have one allele with the Y402H variant of the CFH gene (i.e., heterozygous) is about 68 percent greater than for heterozygous nonsmokers. A current smoker who is homozygous for this gene variant (both alleles have the Y402H variant) has an AMD risk that is 172 percent greater than a homozygous nonsmoker [58]. Dr. Klaver noted that some discrepancies in the observed synergistic effect arise between studies looking at the combined effect of smoking and the gene variants associated with increased AMD risk. These discrepancies may reflect real differences in the subject populations studied, methodological differences, or some combination of both [49, 59].

Dr. Klaver's group has also analyzed the interaction between dietary intake of zinc and AMD risk for Rotterdam study subjects with no, one, or two alleles of the Y402H variant of the CFH gene. Higher levels of zinc in the diet brought the AMD risk for even the homozygous Y402H group close to the risk of the group with no Y402H allele. In the group with the lowest third of zinc intake, the AMD risk with homozygous Y402H was about double the risk for the group with no Y402H allele. The Blue Mountain Eye Study group analyzed its data for an interaction between the Y402H gene variant, smoking, and regular fish consumption. At the 2007 ARVO meeting, the group reported that weekly consumption of fish reduced the risk of late-stage AMD in subjects who were homozygous for Y402H. The analysis also confirmed the synergistic effect of smoking in increasing the AMD risk associated with the Y402H gene variant. These results indicate, Dr. Klaver emphasized, that attention to environmental factors such as not smoking and eating foods that provide healthier levels of protective nutrients are particularly important for individuals with a genetic susceptibility to AMD.

In summing up the implications from all of the above research on AMD risk, Dr. Klaver concluded that recent studies indicate that genetic factors appear to make a larger contribution to an individual's risk of AMD than do environmental factors. Nevertheless, recommen-

dations for a healthier diet and for quitting smoking are still highly relevant to reducing this risk. Although there are discrepancies in some of the study results on nutrition and diet, these appear to be mostly due to differences in study design, outcome selection, and cut-off values, and to lack of sufficient statistical power. Across these studies generally, fish consumption and higher dietary intake (i.e., at levels greater than the RDA but not above recommended ceiling values) of certain antioxidants such as vitamin E and of the mineral zinc appear beneficial. In particular, the deleterious effect of risk alleles on the occurrence of AMD may be ameliorated by healthy lifestyles.

Post-Presentation Discussion

Dr. Allen Taylor asked whether interactions among environmental and genetic factors like those discussed by Dr. Klaver indicate that future studies should have fuller datasets before the data are analyzed for significant results. Dr. Klaver replied that genetic information on study subjects will be essential now to continuing to advance the state of knowledge about environmental risk factors for AMD. This led to general discussion among the symposium participants on the importance of making a compelling argument for designing future studies to address these risk issues, even though this will mean that studies become more costly in resources required and more difficult with respect to sample size and selection. Although many studies of population genetics and AMD are being done, most of them are not designed to account for and gather sufficient data on environmental factors that potentially interact with and influence the expression of genetic factors. This is particularly important for the current circumstances where the most acceptable yet effective interventions to lessen genetically linked disease risks lie in managing the environmental factors that either exacerbate or protect against those risks. Dr. Rohit Varma noted that standardized approaches for collecting data on environmental factors are needed. Otherwise, there will continue to be the kinds of discrepancies between studies, as noted in the presentations on DR and AMD, that make cross-study comparisons and analyses difficult. In contrast, the methods for collecting genetic data are now relatively uniform, making cross-study analyses meaningful and

less difficult. The participants discussed the size of samples needed in epidemiologic studies to ensure adequate statistical power for both environmental and genetic factors of interest. Dr. Sheila West suggested that case-control design methods may need to be added to general population designs to enrich the datasets for factors and combinations of factors that should be investigated.

Nutrition Factors and AMD: AREDS and AREDS2

Dr. Emily Chew

The main topics of Dr. Chew's presentation were (1) the results of the Age-Related Eye Disease Study (AREDS), which included a clinical trial of dietary supplements and an observational study including diet, and (2) the design of the new Age-Related Eye Disease Study 2 (AREDS2). To set the context for these clinical trials, she noted that AMD is currently the leading cause of blindness in the United States. Throughout the developed countries, it is the major cause of blindness in people of non-Latino European ancestry. As people live longer, the number of Americans with vision loss from AMD is expected to increase substantially. Finding ways to slow or prevent the progression of AMD is therefore an important goal for public health reasons, as well as for the quality of life of those affected by this chronic disease of aging.

The pathogenesis of AMD remains uncertain, although oxidative stress in the retina is implicated, as are the gene variations discussed by Dr. Klaver. The retina is especially susceptible to oxidative stress because of the abundant oxygen supply it receives from the blood, the high concentrations of long-chain PUFAs in the retina, and the presence of photosensitizing compounds.

AREDS Clinical Trial and Follow-up Study

AREDS included a prospective natural history study, a randomized, multicenter 6-year clinical trial that concluded in 2001, and a 5-year follow-up study that concluded in 2005. The initial 6-year trial was double-masked, used a placebo control group, and had 4,757 participants. It had

a 2 percent loss to follow-up through its conclusion in 2001. The 5-year follow-up study began with 3,687 of the original AREDS participants and had a 4 percent loss to follow-up.

The participants in the initial clinical trial were divided into four treatment groups for the AMD trial, with all participants receiving an oral supplement, usually administered twice daily. The control group ($N = 1,482$) received placebo. The supplement for the other three groups contained either antioxidants only (500 mg vitamin C, 400 IU vitamin E, and 15 mg beta-carotene), zinc (80 mg zinc and 2 mg copper to reduce the risk of copper deficiency anemia from the zinc supplementation), or both the antioxidant and zinc doses. Participants were recruited into AREDS based upon the severity of their AMD at baseline, assessed according to the following four categories:

- Category 1, designated as having no signs of AMD, had no drusen or just a few small drusen (less than 63 microns). These 1,117 participants without signs of AMD at baseline also served as the cataract-only group for the cataract portion of AREDS.
- Category 2, designated as early AMD, were patients with more extensive small drusen and at most a few intermediate-size drusen.
- Category 3 patients had more extensive intermediate drusen at baseline or had at least one large drusen.
- Category 4, advanced AMD, consisted of patients with either geographic atrophy or choroidal neovascularization in one eye, and large drusen in the other eye. AMD progression was followed for the eye that did not yet have late-stage AMD [60, 61].

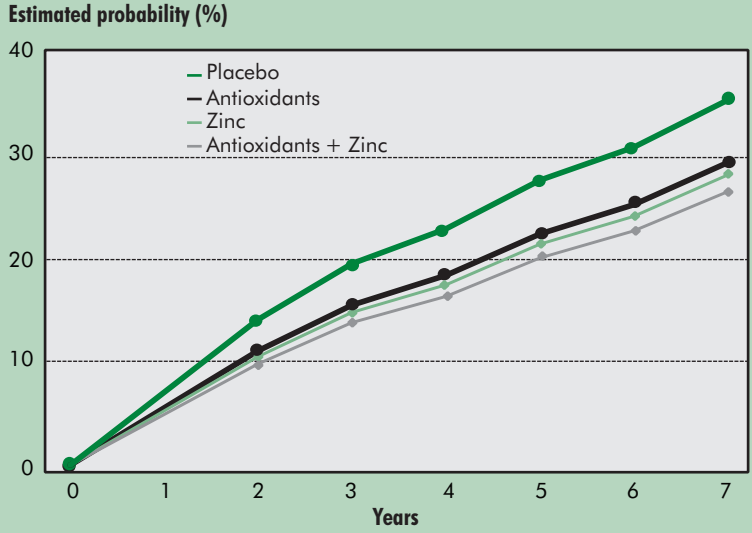
Unlike some other studies, vision loss was not part of the category 2, 3, and 4 definitions in AREDS; all participants in the AMD study had visual acuity of 20/30 or better, although for category 4 this acuity requirement applied only to the eye without late-stage AMD already.

In the placebo treatment group, the rate of progression to advanced AMD for category 2 patients (early AMD) was so low (1.3 percent after 6 years) that Dr. Chew said there is no reason, based on the AREDS results, for people with small drusen or even a few intermediate drusen to consider a dietary supplement specifically for AMD. Category 3

patients had an 18 percent rate of progressing to advanced AMD in 5 years. In category 4, the eye with large drusen progressed to advanced AMD in 43 percent of patients within 5 years.

When categories 3 and 4 are considered together, all three of the treatment groups that received a dietary supplement had reduced rates of progression to advanced AMD. The combined supplement (antioxidants plus zinc) had the largest protective effect, reducing the rate of progression to late-stage AMD by 25 percent in category 3 and 4 patients (from 28 percent progression in those receiving placebo to 20 percent progression in the treatment group receiving the combined supplement). Figure 9 shows the rates of progression by treatment category.

Figure 9. Long-Term Rate to Advanced AMD for AREDS Severity Categories 3 and 4 Combined



Note: After 5 years, the antioxidants + zinc treatment group had 25 percent lower risk of progression to advanced AMD than the placebo group. For placebo versus antioxidants + zinc, $p < 0.01$; for placebo versus antioxidants alone, $p < 0.01$.

Source: AREDS investigators; data courtesy of Dr. Emily Y. Chew.

During the 5-year follow-up study, all the category 3 and 4 participants were urged to take the combined supplement. After 10 years from the original AREDS baseline, the treatment effect persisted, with a 27 percent reduction in the risk of progression to advanced AMD for the category 3 and 4 patients who had received the combined supplement during the clinical trial. In response to a question about the rate of progression in patients who did not stay on the treatment, Dr. Chew and Dr. West discussed the difficulties with analyzing data for on-and-off treatment. On the compliance issue, Dr. Chew suggested that the size of the original pills may have been a factor, as well as some patients' difficulty in tolerating the supplement, which can cause nausea and gastrointestinal upset for about 1 percent of subjects. She noted that similar problems with sustaining compliance with a preventive treatment regimen had occurred with attempts to sustain tight glycemic control among participants in the Diabetes Control and Complications Trial.

Meta-analyses Indicating Adverse Effects of Vitamin E

Because the AREDS antioxidant formulation includes 400 IU of vitamin E, the AREDS Research Group became involved in the controversy over a 2005 meta-analysis of adverse effects from supplements in 19 clinical trials. This meta-analysis reported an increased mortality risk for adults taking a 400 IU vitamin E supplement [62]. In response to this report, Dr. Chew and Dr. Traci Clemons conducted their own meta-analysis of three randomized clinical trials (total $N = 15{,}162$), including AREDS, in which the vitamin E dose was 400–440 IU. They found that the pooled group receiving vitamin E had slightly lower mortality than the pooled placebo group (801 deaths out of 7,564 taking vitamin E supplement, 806 deaths out of 7,598 taking placebo) [63]. There were also many comments to the editors of *Annals of Internal Medicine* about methodological flaws in the first meta-analysis.

A subsequent meta-analysis by different authors analyzed 68 clinical trials, including AREDS, of antioxidants to reduce disease risk. The antioxidants in each study included one or more of the following nutrients: beta-carotene, vitamin A, vitamin C, vitamin E, and selenium. When

all low-bias and high-bias trials of antioxidants were pooled together, there was no significant effect on all-cause mortality (relative risk, 1.02; 95 confidence limits, 0.98–1.06). For the set of low-bias trials, after excluding trials with selenium, the meta-analysis found that vitamin E singly or combined with beta-carotene and vitamin A, was associated with increased mortality at a statistically significant level (relative risk, 1.04; 95 percent confidence limits, 1.01–1.07). The authors concluded that treatment with beta-carotene, vitamin A, and vitamin E may increase mortality [64]. Dr. Chew noted, however, that there were methodological issues with this meta-analysis as well, such as not controlling for smoking in several of the trials that had large numbers of deaths from smoking-associated causes.

Dr. Chew suggested that, given the continuing issues over appropriate methodology for meta-analyses of quite disparate clinical trials using antioxidants for diverse treatment effects, a more important consideration may be the mortality experience of AREDS participants. After adjusting for age and gender, the four treatment groups in the AREDS clinical trial had the following mortality rates: placebo group, 10.9 percent; antioxidants alone, 10.8 percent; zinc alone, 8.5 percent, and combined antioxidants and zinc, 9.2 percent. The AREDS intervention with antioxidants and zinc thus was not associated with increased mortality relative to placebo (relative risk, 0.87; 95 percent confidence limits, 0.71–1.06) [65]. This analysis for mortality in the AREDS study population has been repeated during the follow-up study. Dr. Chew reported that the reduced mortality rate with zinc-only treatment has continued through the follow-up period and is statistically significant (relative risk, 0.81; 95 percent confidence limits, 0.71–0.94). The clinical significance of this reduced mortality is unknown, and Dr. Chew suggested that further studies will be required to understand it.

Conclusions from AREDS

Dr. Chew drew three major conclusions from the results of the AREDS clinical trial and the follow-up study to date:

1. The beneficial effects of antioxidants and zinc in reducing the rate of progression to advanced AMD from intermediate AMD

(i.e., from AMD category 3 or 4 at the beginning of treatment) persist at 10 years of follow-up.

2. No beneficial treatment effects were found for progression of baseline category 1, 2, or 3 to early or intermediate AMD (that is, to category 2 or 3 at follow-up).
3. There was no increase in mortality for AREDS participants who received the AREDS formulation.

AREDS Observational Study of Dietary Nutrients

During the AREDS clinical trial and follow-up, a daily food intake questionnaire was used to gather data on diet. Based on analysis of the questionnaire data, dietary levels of a number of nutrients of interest were estimated, including lutein/zeaxanthin and PUFAs. For example, five levels of intake of lutein/zeaxanthin were defined, and the relative risks (odds ratios) for progression to neovascular AMD or to geographic atrophy AMD were estimated. These analyses, which controlled for a number of known risk factors and other associations with AMD, showed statistically significant associations of higher intake of lutein/zeaxanthin with reduced risk of AMD progression to advanced AMD (either neovascularization or geographic atrophy) but not for progression to earlier AMD stages (early AMD or intermediate AMD) [66]. Other observational studies have also found that high dietary intake of lutein/zeaxanthin is protective for neovascular AMD. The AREDS dietary intake study found an association of higher serum levels of lutein/zeaxanthin with reduced risk of progression to advanced AMD.

The AREDS dietary study examined dietary intake of omega-3 long-chain PUFAs in the same way [67]. The analysis of the questionnaire data showed statistically significant decreased risk of neovascularization or geographic atrophy for higher levels of total dietary omega-3 PUFAs and for two specific fatty acids in this group: docosahexaenoic acid (DHA) and eicosapentaenoic acid. The types of food associated with higher levels of these omega-3 fatty acids included broiled or baked fish but not fried fish. At least one serving of broiled or baked fish per week gave a dietary intake sufficient to reduce risk of progression to advanced AMD, compared with not eating broiled or baked fish (table 4). By con-

Table 4. Association of Broiled or Baked Fish Intake with Risk of Neovascular AMD versus No Drusen (Controls)

Servings of fish	Odds ratio[a]	95% confidence interval
Less than 1 per month	0. 96	0. 7 – 1. 3
1 to 3 per month	0. 81	0. 6 – 1. 1
1 per week	0. 77	0. 5 – 1. 1
More than 1 per week	0. 64	0. 4 – 1. 0

[a]As compared with no intake of fish.

Source: [67].

trast, higher dietary levels of arachidonic acid, an omega-6 PUFA, were associated with an increased risk of progression to neovascular AMD. (The odds ratio for the highest quintile of dietary intake versus the lowest quintile was 1.54, with 95 percent confidence limits from 1.1 to 2.3.) Dr. Chew agreed with Dr. West's earlier comment that the levels of specific nutrients may not be as important as overall dietary patterns.

Design and Preparation for AREDS2

In general, Dr. Chew said, the design for AREDS2 is based on the results from the original AREDS observational study of diet as well as on the AREDS clinical trial results for dietary supplements. The planned dietary supplements include lutein (10 mg) and zeaxanthin (2 mg) and 1 g of long-chain PUFAs (350 mg DHA, 650 mg eicosapentaenoic acid). This multicenter randomized clinical trial will involve academic centers and community clinics located across the United States. Unlike the first AREDS, clinics in the South and West will be better represented. Major ethnocultural minorities, including African Americans and Latino Americans, may be better represented than in AREDS. To be included in the study, patients will need to have at least large drusen in both eyes. They can have advanced AMD in one eye and large drusen in the fellow eye. The study eye need not have evidence of drusen if it has definite geographic atrophy not involving the center of the macula.

All patients will be offered an AREDS-type supplement. All participants will receive 500 mg vitamin C and 400 IU vitamin E. Patients who are active smokers will have no beta-carotene in their supplement; nonsmokers will receive 15 mg of beta-carotene or no beta-carotene. At each level of beta-carotene, there will be another division into a zinc dose of either 80 mg zinc oxide (with 2 mg cupric oxide) or 25 mg zinc oxide (with 2 mg cupric oxide). As of June 2007, 1,800 patients had been randomized to the treatment groups of the trial, with a total of 4,000 participants planned.

The primary outcome for AREDS2 is progression to advanced AMD. Secondary outcomes are progression to moderate vision loss and disease progression on a simplified AMD severity scale developed during the first AREDS. For the cataract portion of the study, the outcomes are time to cataract surgery and progression of lens opacity. The National Heart, Lung, and Blood Institute is contributing funding for evaluating effects of the AREDS2 dietary supplements on cardiovascular morbidity and mortality. The National Institute on Aging is doing the same for evaluation of the effects of the supplements on cognitive function. Current information on AREDS2 is available online at www.areds2.org.

Post-Presentation Discussion

Dr. Chew and Dr. Sayoko Moroi discussed the exclusion of patients with glaucoma from AREDS2 because of concerns that the treatment effect for AMD might be masked. They also discussed ways in which the original AREDS data could be used to examine whether the AREDS dietary supplements had any effect on IOP or on glaucoma progression. In this regard, Dr. Chew described plans to analyze the AREDS data for trends related to glaucoma indicators, but she noted that the effects on the data of AREDS patients who started using or changed IOP-lowering medications during the trial would complicate the analysis. Nevertheless, she said, the AREDS and AREDS2 study populations have demographic characteristics that make them potentially useful for investigating glaucoma risk and protective factors. Although the patient health and diet questionnaire already contains some questions about symptoms of dry eye syndrome, Dr. Chew thought it may need to be expanded to address

issues such as those Dr. Sheila West had raised in her presentation about distinguishing the forms of dry eye and identifying external factors (such as iatrogenic dry eye).

Dr. Allen Taylor asked about the implications of the observed continuation of a treatment effect in AREDS beyond the 6 years of the initial clinical trial and through the 10 years including follow-up. He contrasted the environment of the retina with that of the crystalline lens, where some of the proteins are very long-lived. Given the slow turnover of lens proteins, a long-term intervention by dietary supplementation, as in AREDS, is likely to be necessary to see an effect. However, once the intervention has begun to make a difference, one would expect the beneficial effect to endure for a long time as well. Given the much more rapid turnover of retina components, the biological basis for a long-term continuing effect in the retina is less clear. Dr. Chew replied that interaction of the dietary and nutritional supplement factors with genetic factors is an area of interest for understanding the biological basis of the enduring effects observed in AREDS. She described preliminary results indicating that zinc intake may be important because zinc may be associated with CFH production or effectiveness, thereby promoting anti-inflammatory factors linked to genetic differences such as those described by Dr. Klaver.

Dr. West commented that the AREDS baseline AMD categories 3 and 4 were very diverse with respect to phenotypes included in each class. The dietary intervention may in fact have limited disease progression for some of these phenotypes, even though the effect would not have been identified by the AREDS outcome measures. She suggested trying to distinguish among phenotypes at the end of the initial clinical trial by careful examination of the fundus photographs at 6 years, then examining how these phenotype subgroups progressed or failed to progress (e.g., in increased size and number of large drusen) during the follow-up period out to 10 years. Dr. Chew described how the AMD severity scale developed during AREDS could help with the types of investigations Dr. West and Dr. Taylor were suggesting.

Dr. Klaver said that the rates of AMD progression reported from AREDS are similar to the overall rates found in the Rotterdam study

progression. However, when the Rotterdam population is also stratified by age, the progression rate is much higher for the group 75 years of age and older than for younger groups with the same baseline AMD classification.

Dr. John Hetherington asked about the appropriate guidance for clinicians, given all of the AREDS results to date. Dr. Chew agreed that this was an important issue. She said that, for patients who are the equivalent of AREDS category 1 or 2 in AMD symptoms, even if they have older relatives with AMD, there is no current justification for them to take an AREDS-type supplement in addition to maintaining a diet that is generally healthful. In response to Dr. Moroi's question about stratification of the AREDS work on C-reactive protein as a marker for AMD risk [68], Dr. Chew said the sample of AREDS patients for which there were blood samples was too small to do further stratification in the analysis. The plan for AREDS2 is to collect blood samples from all the participants.

Nutrition, Environment, and Retinitis Pigmentosa

Dr. Theo van Veen

Dr. van Veen's presentation began with a summary of current knowledge about disease progression in retinitis pigmentosa (RP), particularly the antecedents and consequences of photoreceptor cell death. He described recent findings in animal models for RP on the role that oxidative damage to photoreceptor DNA plays in the disease process. A key concept is that saving the rod photoreceptors, which in RP are generally affected first, saves the cone cells. In the context of this view of RP pathogenesis, Dr. van Veen discussed recent work and prospects for the use of nutrition supplements to slow RP progression and thereby prevent or delay serious vision loss.

Interplay of Genetic Factors and Oxidative Damage in RP Pathogenesis

In most forms of RP, a gene mutation that affects the rod photoreceptors is the underlying genetic factor in the etiology of the disease. This

gene defect leads to the death of rod photoreceptors and subsequently to the death of cone cells, even though the cone cells do not express the mutated gene. Gene therapy is one approach to treating RP. A large number of the genes whose mutations are responsible for individual RP genotypes have already been identified, and two gene therapy clinical trials for one genotype are under way. One constraint on gene therapy, however, is that only an estimated half of all the genes whose mutations cause different forms of RP have been identified. Thus, about half of all RP patients do not yet have potential access to a gene replacement approach to treating their disease.

A more general approach, which does not require identifying and treating the specific genetic factors in a given disease genotype, is to protect the photoreceptors from cell death. This can be done through the use of neuron-survival or neurotrophic agents. One such trial of a neuroprotective agent, ciliary neurotrophic factor, was recently completed.[4] Another general approach to protecting the photoreceptors may be the use of antioxidant supplements that protect against the oxidative damage and subsequent photoreceptor cell death that have been demonstrated to occur in the retinas of a number of animal models for RP. A clinical trial of antioxidants for photoreceptor protection is now under way.[5]

Using photomicrographs of retinal degeneration in a cat model for a form of RP caused by a mutation in the CEP290 gene [69], Dr. van Veen has shown how even partial loss of photoreceptors is accompanied by abnormal migration of Mueller glial cells into the outer nuclear layer and restructuring of the normal pattern of cellular organization across all retinal layers, not just in the photoreceptor layer. In human RP as in this cat model, the photoreceptors die by some form of apoptosis (programmed cell death). Among the conditions that can induce apoptosis is hyperoxia, an extracellular environment higher than usual in oxygen

[4]The trial name is "Evaluation of Safety of Ciliary Neurotrophic Factor Implants in the Eye." Details are available online at the http://clinicaltrials.gov website. The study identifier is NCT00063765.

[5]The trial name is "OT-551 Antioxidant Eye Drops to Treat Geographic Atrophy in Age-Related Macular Degeneration." The study identifier on the http://clinicaltrials.gov website is NCT00306488.

and in reactive oxygen species. Several studies in animal models of retinal degeneration (Royal College Surgeon rat [70], monkey [71], and Abyssinian cat [72]) have shown that the outer nuclear layer becomes hyperoxic in the progression of the disease. Specifically, once photoreceptors begin dying in a retinal area, there is no longer sufficient metabolic demand in the outer layer to use up the abundant oxygen supplied via the choroid.

Deleterious reactive oxygen species are produced in this hyperoxic environment both metabolically and by the action of light on photoreactive compounds in the retina. They can damage cellular structures, particularly the outer segments of photoreceptors, by peroxidation of membrane lipids and other reactions. These reactive oxygen species also damage photoreceptor cell DNA, triggering apoptosis. Dr. van Veen's group and others have examined nine different animal models for RP-like retinal degeneration (pig model for RP, Abyssinian cat, four mouse models, and three rat models). In all of them, oxidative damage to photoreceptor DNA was found.

One hypothesis to explain the death of cone photoreceptors after the death of large numbers of rod photoreceptors is that the increasing hyperoxia in the outer layer, due to the loss of increasing numbers of rod photoreceptors, eventually damages the cone photoreceptors enough to cause their death as well. Support for this hypothesis comes from a study of progressive oxidative damage to the proteins and DNA of cone cells in the transgenic pig model for RP. As more of the rod receptors were lost, the amount of oxidative damage to the cones increased [73].

Nutrient Supplements and RP

Studies on animal models and clinical trials have shown that vitamin A supplementation can slow photoreceptor degeneration in animal analogues of RP and in human RP patients. The results of controlled trials indicate that nutritional interventions, including vitamin A palmitate and fish rich in omega-3 PUFAs, slow progression of the disease for many RP patients [74, 75].

The specific form of RP may determine whether vitamin A supplementation is beneficial or harmful. A 2007 review article noted

that many retinal dystrophies (including forms of RP) have been tied to defects in the light transduction sequence that changes the chromophore in opsin from 11-*cis*-retinaldehyde to all-*trans*-retinaldehyde [74]. In normal vision processing, the all-*trans*-retinaldehyde is quickly recycled back to the 11-*cis* isomer for reuse and the reactive byproducts of the opsin cycle are removed. The retinal dystrophies discussed in the review can be divided into two groups: in one, the defect impairs synthesis of the visual chromophore; in the second group, the defect leads to accumulation of cytotoxic products derived from all-*trans*-retinaldehyde. Vitamin A supplementation is likely to be beneficial for the first group. For the second group, inhibiting the visual cycle, including *limiting*, rather than supplementing, the supply of vitamin A, is an option for pharmacologic intervention. The authors also noted that gene therapies designed to address the underlying gene defect(s) that interfere with normal visual processing have been successful in animal models for defects in both groups [74].

In two recent studies of whether antioxidant supplementation would reduce cone cell death in animal models for RP, Campochiaro and his coworkers tested four antioxidants in three mouse models [76, 77]. The antioxidants were vitamin E (α-tocopherol), vitamin C (ascorbic acid), α-lipoic acid, and MnTBAP [manganese(III) tetrakis(4-benzoic acid) = porphyrin, a synthetic metalloporphyrin]. In the first study, rd1 mice received a daily injection of each antioxidant alone or a mixture of all four. Both vitamin E and α-lipoic acid alone, as well as the antioxidant mixture, promoted cone survival compared to controls. Neither the vitamin C nor the MnTBAP alone had a protective effect. Promotion of cone survival was assessed by both a reduction in oxidative damage in cone photoreceptors and by preservation of cone function (measured by photopic electroretinograms) [76]. The second study showed that the antioxidant mixture promoted cone survival and preserved cone function in two other mouse models for RP, one a model for slowly progressing RP, the second a model for rapidly progressive dominant RP [77].

Dr. van Veen noted that several older studies of vitamin E supplementation reported adverse effects either in RP patients [78] or as an observed association of new RP development with vitamin E supplemen-

tation to treat a disorder caused by a mutation in the gene for a protein that transfers α-tocopherol [79]. Dr. van Veen suggested that caution should be exercised in using vitamin E supplements for RP patients until further investigation clarifies the situation.

Next, Dr. van Veen described research by his group on antioxidant treatment to reduce oxidative damage in the rd1 mouse model for RP [80]. The antioxidants studied were lutein and zeaxanthin, glutathione, and α-lipoic acid. For an in vitro study, retinal organ explants were cultured for 13 days, then prepared for histologic examination to assess the survival of photoreceptors in the outer nuclear layer. The explants cultured with either one antioxidant or the mixture were compared with a similar explant from the contralateral eye, which had been cultured in medium with vehicle alone (no antioxidant). The cultures with antioxidant had 25 percent more photoreceptors than the untreated controls. In addition, they had 40 percent fewer TUNEL-positive cells and 35 percent fewer Avidin-positive cells, indicating partial protection of the cells from oxidative damage to proteins and DNA.

For the in vivo study, the antioxidants were administered at a dosage for which no toxic effects have been reported. Lutein/zeaxanthin at 0.35 mg/kg, glutathione at 3.5 mg/kg, and α-lipoic acid at 1.5 mg/kg were added in combination to the treatment group's diet. After 11 days, the numbers of rows in the outer nuclear layer were the same for the treated and untreated mice. However, there were 60 percent fewer cells with protein damage and 40 percent fewer cells with DNA damage in the treatment group. After 17 days, there were more surviving cells in the outer nuclear layer of the peripheral (27 percent more) and mid-peripheral (32 percent) retinal areas of the mice that received the antioxidant supplementation compared with the untreated controls [80].

Summarizing the results from the in vitro and in vivo studies by his group, Dr. van Veen said that a significant protective effect was observed with combinations of antioxidants. Single antioxidant supplements had a small protective effect that was not statistically significant. Although the observed protective effect may seem small, even a one-day delay in the amount of retinal degeneration in an rd1 mouse represents a slowing of the rate of degeneration by 20–25 percent. Preliminary studies (not

yet published) on the rds mouse have also found a protective effect from a combination of antioxidants with respect to oxidative damage to both cell DNA and cell survival. A clinical study is now in progress to investigate this effect in humans.

General Conclusions on Progression in RP and Other Retinal Degenerative Diseases

The general picture of disease progression in many forms of RP and other diseases of retinal degeneration, Dr. van Veen said, is that oxidative damage mediates a process of progressive photoreceptor die-off. In nine different animal models for RP, some involving spontaneous mutations and others with transgenic animals, oxidative damage to photoreceptor DNA has been found. The rod photoreceptors begin dying first, as accumulated cell damage initiates apoptosis. The cone photoreceptors begin to die after many of the rod cells have been lost. Therefore, if the rod photoreceptors can be rescued, not only can vision in dimly lit conditions be preserved but the hyperoxia that ultimately leads to cone photoreceptor death can also be prevented or at least lessened. For humans, cone survival is especially important since the cones are needed for daylight (bright light) vision. Moreover, the high concentration of cones in the macula provides our central, sharp, and color vision. Prior to the point where cell damage is so great that apoptosis is initiated, cells—including both rod and cone photoreceptors—do have mechanisms to repair lesser levels of oxidative damage.

In this context, it appears that certain antioxidants can save the cone photoreceptors by enhancing defenses against oxidative damage. The work by Dr. van Veen's group [80] and that by Dr. Campochiaro [76, 77] with combinations of antioxidants indicate that, by using a combination of agents that scavenge the reactive oxygen species formed in the high-oxygen conditions of the outer retina, both rod and cone photoreceptors can be protected sufficiently to slow disease progression to severe vision loss. Although such treatment with antioxidants can slow but not halt the loss of photoreceptor cells completely, it may buy time for other, more effective treatments to be developed and made available. And even with a new and effective therapy that addresses other steps in

the disease sequence, such as growth factors that block the initiation of apoptosis, antioxidant treatment may still be useful as an adjunct therapy to deal with the continued high-oxygen levels in the outer retina.

Post-Presentation Discussion

Dr. Chader commented that the decrease in rate of photoreceptor loss observed by Dr. van Veen and his group with combinations of antioxidants seems comparable to the 25 percent decrease in rate of AMD progression observed in AREDS (for categories 3 and 4 AMD at baseline). He asked whether this similarity might represent a constraint on antioxidant protection related to some pathway or other factor that is common to both the AMD and RP pathologies, as opposed to just a coincidence. Dr. Chew noted that some researchers think that AMD begins as a disease of the rod photoreceptors before it progresses to the cones in the macula, similar to the progression described by Dr. van Veen for RP models. If so, then antioxidant treatments that have shown protective effects for these two diseases may be intervening at the same stage of a common pathway. Dr. van Veen said that he has not examined AMD-diseased tissue to determine whether there is DNA damage in the photoreceptors similar to what he has observed in RP models. However, shortly after his symposium presentation, a report demonstrating oxidative DNA damage in photoreceptors of AMD patients was published [81].

The symposium participants continued discussing various lines of research linking oxidative damage to dysfunction of cellular proteins and DNA, effects of antioxidants in limiting such damage, and the possibility of a pathway common to multiple degenerative diseases of the retina. An intriguing question addressed by the participants was "Within the cell, where does the precipitating damage occur? In the mitochondria, the nucleus, or elsewhere?" Although the answer to this question is unknown, the participants agreed the question is extremely important, as even more potent inhibitors of oxidative damage and apoptosis might be uncovered once the intracellular loci of oxidative damage are identified.

Dr. Sayoko Moroi asked whether, given the widespread use of ginkgo biloba herbal supplements in Europe, notably in Germany, there had

been any research on the effect of such supplements in any of the animal models for RP that Dr. van Veen had described. Dr. Chader mentioned that, although there have been sporadic reports in the scientific literature at least back to the 1980s on protective effects of ginkgo biloba on ocular conditions such as AMD and normal-tension glaucoma, he knew of none specifically on RP. Dr. Nicolas Bazán said that gingko biloba extract contains a large number of potentially active compounds; it is a very complex mixture. It may contain neuroprotective factors as well as antioxidants. A major obstacle to conducting scientific studies of gingko biloba or comparing across studies, he added, is that the extracts differ greatly in composition depending on how they are prepared. Stability is another difficulty. Dr. van Veen described cell culture studies and noted the difficulty in trying to isolate the active factor(s) of such extracts.

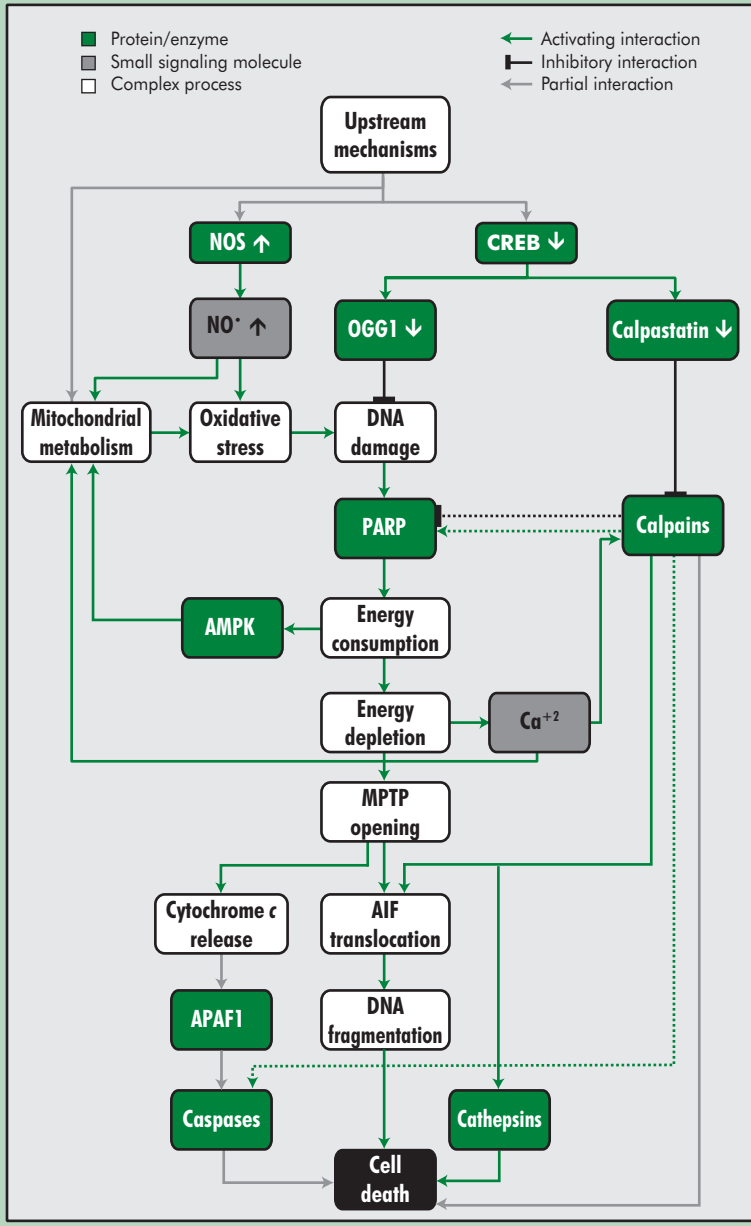
Dr. John Hetherington noted the current research interest in damage to glial cells as a key step in the pathway to optic nerve damage in glaucoma. He asked if there was any evidence connecting glial cell damage with photoreceptor loss in AMD or RP. Dr. van Veen and Dr. Bazán said there was evidence of glial cell involvement in both AMD and RP. Dr. Chader noted that the dominant paradigm for pathogenesis in the retinal degenerative diseases had shifted from an earlier emphasis on damage caused by the oxidative products of photoreactions to a view that the pathways for different diseases could be diverse because of the differences identified in genetic risk factors. Now, he said, there seems to be a renewed interest in possible common stages in the disease pathway, a final-stage or late-stage commonality in the pathways for a number of diseases, reflecting the consequences of oxidative damage. These stages would operate after the initiating stages of a particular disease—for example, the effects on a protein expressed by a gene mutation. This part of the disease sequence may be similar in multiple diseases even though the initiating conditions are heavily influenced by genetic factors specific to a disease or even a single genotype of a disease. If a common element in disease pathway does exist, then, as Dr. van Veen and others have suggested, intervening at this stage with antioxidant supplementation could be a noninvasive, relatively inexpensive, and patient-friendly way to slow disease progression even if the initiating conditions persist.

Such pathways involving oxidative DNA damage could function, according to one of Dr. van Veen's collaborators, Dr. Francois Paquet-Durand, as shown in figure 10. In addition to hyperoxia, oxidative stress is triggered by upstream mechanisms that could, for instance, include genetic defects or lack of neurotrophic support. These factors could directly affect mitochondrial metabolism, leading to increased production of reactive oxygen species and oxidative stress. Elevated activity of nitric-oxide-synthase (NOS) produces nitric oxide radicals (NO•), which affect mitochondrial metabolism but also directly contribute to oxidative stress. Down-regulation of the transcription factor cyclic-AMP-response-element-binding protein (CREB) leads to down-regulation of CREB target genes including 8-oxoguanine DNA glycosylase, an enzyme critical for the repair of oxidatively damaged DNA, and calpastatin, the endogenous inhibitor of calpain-type calcium-activated proteases.

These events all cause an increase in oxidatively damaged DNA and hence activation of enzymes engaged in DNA repair, such as poly-ADP ribose polymerase (PARP). Excessive activation of PARP, however, increases energy consumption, which causes activation of AMP-activated kinase and increases mitochondrial activity. These consequences also cause additional oxidative stress. Eventually, failure of mitochondrial metabolism to cope with rising demand leads to energy depletion, mitochondrial depolarization, opening of the mitochondrial permeability transition pore (MPTP), and secondary influx of calcium ion. The calcium ion influx affects mitochondrial metabolism negatively and activates calpain-type proteases, which may already be more susceptible for activation due to down-regulation of calpastatin. Calpains have a large number of substrates including PARP, apoptosis inducing factor (AIF), and caspases. They may also mediate activation of cathepsin-type proteases and may induce cell death through other mechanisms that are still not fully understood.

Opening of the MPTP allows for translocation of AIF from the mitochondria to the nucleus. AIF translocation may, however, also be induced by calpain activity independently of MPTP opening. In the nucleus, AIF activates specific endonucleases that cause DNA fragmentation, effectively killing the cell. Release of cytochrome *c* from

Figure 10. Molecular Pathways from Oxidative Stress to Photoreceptor Degeneration



Note: See text for abbreviations. Dotted lines indicate uncertain interactions.

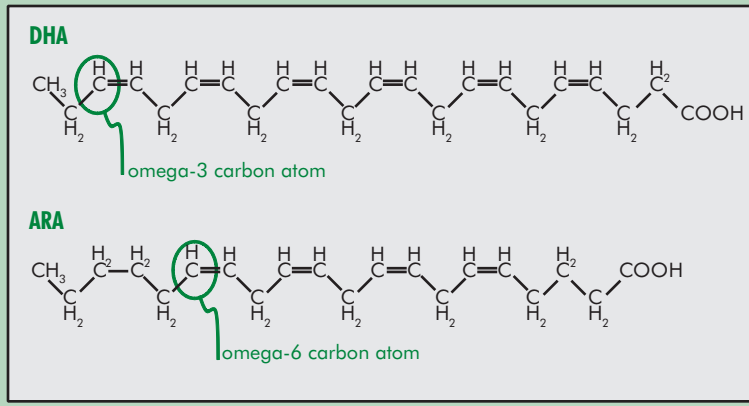
mitochondria after MPTP opening may lead to formation of apoptotic protease-activating factor 1 and subsequent activation of caspases. Since these processes form part of the classical apoptotic cascade, they depend on protein biosynthesis and an intact energetic status. They are therefore unlikely to be involved in cell death resulting from oxidative stress.

Role of Dietary Fatty Acids in Brain and Eye Development

Dr. Eileen Birch

Fatty acids are lipids that are essential components of all cells (e.g., cell membranes). They are basically composed of chains of carbon atoms, in which the chain can vary in length and may have double bonds (unsaturation) linking some of the carbons. One of the best known and studied is DHA, a long-chain (22 carbons), unsaturated (6 double bonds) fatty acid (figure 11). The importance of DHA for normal brain functioning and vision is suggested by its elevated levels in the brain and retina, where it represents 30–40 percent of total fatty acids, compared with just

Figure 11. Docosahexaenoic Acid and Arachidonic Acid



Note: DHA is an omega-3 polynunsaturated fatty acid with 22 carbon atoms and 6 double bonds; ARA is an omega-6 polyunsaturated fatty acid with 20 carbon atoms and 4 double bonds.

2–4 percent in the cell membranes of the rest of the body. Studies during the 1970s of nursing primate infants found that depriving them of DHA caused irreparable changes in the retina and brain. At that time, the infant formulas used for bottle-feeding human babies contained no DHA. These considerations led Dr. Birch and her colleagues to investigate ways of providing dietary DHA to nursing infants. The human brain increases 260 percent in weight during the last 3 months before birth, then grows another 175 percent from its size at birth during the first year of infancy [82]. The number of neural synapses in the visual cortex of the infant brain increases more than 5-fold in the first 4 months of infancy [83]. During this preterm and early infancy period, out to 2 years, the amount of DHA in the brain also increases rapidly [84].

Before birth, the fetus is supplied with preformed DHA from its mother by preferential transport across the placenta. After birth, nursing infants receive preformed DHA from breast milk or from infant formula that has been fortified with DHA. Since 2002, infant formulas fortified with DHA and arachidonic acid have been available in the United States. (The structure of ARA, an omega-6 PUFA; is shown in figure 11.) As infants are weaned, they can also get DHA from solid foods that contain it naturally or that have been fortified with it. A backup system to these direct sources of preformed DHA is metabolic conversion of α-linolenic acid to DHA, but the conversion rate is too low to meet the needs of the rapidly growing brain and retina [85, 86].

Clinical Trials of DHA-Fortified Formula Compared with Nonfortified Formula and Human Milk

Dr. Birch described the general structure of the 12 prospective, double-blind, clinical trials that she and her colleagues at the Retina Foundation of the Southwest have conducted over a number of years to compare measures of brain and vision development in infants and children receiving different amounts of dietary DHA. One group of infants in these studies was bottle-fed with commercial infant formula. A second group was fed an experimental formula fortified with DHA and ARA. The group receiving commercial formula served as the control group. DHA intake and developmental outcomes for the two trial groups were also

compared with DHA intake and outcomes for children from the same sociocultural context who were breastfed—the breastfed group provided the benchmark ("gold standard") for healthy dietary intake.

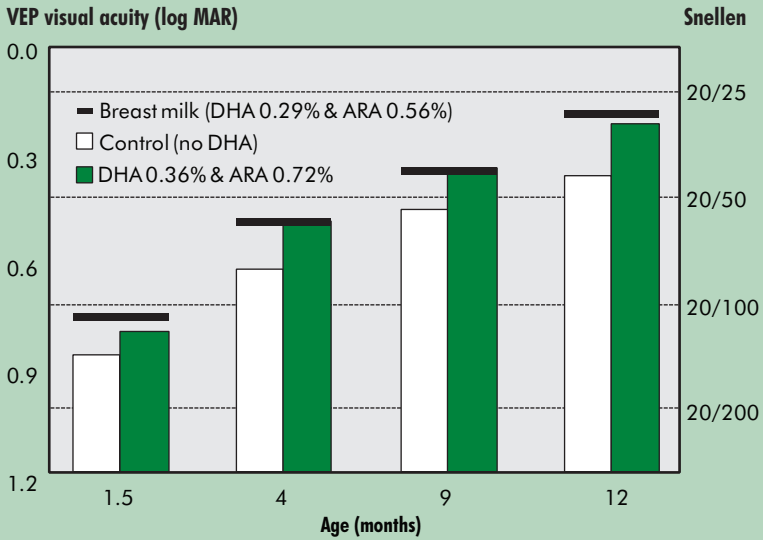
An important environmental variable is that the amount of DHA in human milk as a percentage of total fatty acids varies substantially depending on the mother's diet. In a fishing village in China, DHA in human milk was measured at 2.79 percent of total fatty acids [87]; studies in the United States have found percentages of 0.12 and 0.29 percent [88, 89]. Worldwide, the median value is about 0.35 percent.

Because tissue samples cannot be taken from the brain and retina of live children for testing, the DHA concentration in red blood cell membranes is used as a surrogate indicator. In general, the clinical trials comparing breastfed infants with infants receiving different amounts of DHA in formula show that the level of DHA in red blood cell membranes accurately reflects the relative level of dietary DHA [88, 90].

With respect to visual development outcomes, both rod electroretinograms and two measures of visual acuity (visual evoked potential [VEP] and Teller acuity cards) were more mature for premature infants if they were bottle-fed with formula fortified with DHA than if their formula was unfortified [91, 92]. All randomized clinical trials have shown a benefit for visual outcome from DHA supplementation of formula for infants born prematurely.

For infants born at full term and bottle-fed with DHA-fortified formula, VEP visual acuity was equivalent to breastfeeding and was significantly better than visual acuity for infants bottle-fed with unfortified formula (figure 12) [89, 93]. Furthermore, continuing either breastfeeding or DHA-fortified bottle feeding for a longer duration after birth, up to 12 months, resulted in improved visual acuity at 12 months [94]. Feeding DHA-fortified solid food during the first year of infancy, if infants are weaned at 4–6 months, is also effective in providing the DHA needed for optimal visual acuity development [95]. Dr. Birch briefly reviewed additional assessments by her group of cognitive development outcomes, including mental development index, psychomotor development index, language and school readiness measures, and executive function outcomes. In general, the same patterns occur for these brain development

Figure 12. Visual Acuity of Full-Term Infants Fed Formula with Preformed DHA in 12-Month Feeding Study



Note: Visual acuity of infants fed formula with DHA was similar to that of infants on breast milk. For ages 1.5 and 4 months, the difference between control and DHA-fortified groups is significant at $p = 0.01$; for ages 9 and 12 months, significance is $p < 0.001$.

Source: [93].

outcomes as for the visual acuity outcomes: DHA-fortified infant formula or breastfeeding produces better outcomes than feeding with non-fortified formula.

Meeting Recommended Levels of DHA Intake for Bottle-fed Infants and for Pregnant and Nursing Women

Next, Dr. Birch reviewed the recommendations by several expert panels for the DHA level in formula for preterm and term infants. Most but not all of the commercial infant formula products marketed in the United States meet at least the recommended minimum supplementation, measured as milligrams of DHA per 100 calories of nutrition intake. Unfortunately, the average daily DHA intake for pregnant and nursing women

in the United States is less than a fifth of the recommended daily intake. Given the importance of adequate maternal DHA intake as the principal source of DHA for the fetus and for breastfed infants, this is a cause for concern. In both the United States and Canada, dietary DHA as a percentage of total fatty acids in the diet declined in the decade from 1988 to 1998. Fatty fish, including canned salmon and tuna as well as broiled or baked fresh fish, are among the best sources of DHA (see table 2, p. 38).

Some fish that are excellent sources of DHA are nevertheless not recommended for pregnant or nursing mothers because of concerns about mercury, PCBs (polychlorinated biphenyl compounds), and other toxic chemicals that accumulate in fish at the top of marine food chains. After advisories recommending against eating some fish were issued by the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency, consumption of fish (including canned tuna) by pregnant women has declined further [96]. Nonfish DHA supplements are available as softgel capsules from several commercial suppliers. Taking one capsule per day typically brings the DHA intake up to near the recommended level and increases the DHA content in breast milk. Several studies have confirmed that use of these supplements produces measurable improvements in indicators of infant cognitive development [97, 98].

Summarizing the key points of her presentation for the symposium theme, Dr. Birch said that DHA accumulation in the retina and brain is rapid from the third trimester of fetal development through the second year of infancy. A dietary source of preformed DHA is necessary to meet the requirements of the developing brain and retina during this time. DHA-supplemented infant formulas have been linked to visual acuity and cognitive development outcomes that are comparable to outcomes for breastfed infants and that are significantly better than outcomes for infants fed nonfortified formula. Formulas with greater than 0.25 percent DHA (as a percentage of total fatty acids) routinely provide for optimal neurodevelopment. Breast milk DHA varies, depending on maternal intake of DHA; increasing maternal DHA intake to recommended levels is associated with better developmental outcomes.

A public health issue that should be addressed, Dr. Birch continued, is determining the optimal levels of DHA in infant formula and in dietary supplements for pregnant and nursing women. A closely related issue is the ratio of DHA to ARA in PUFA supplementation. The current 1:2 ratio by weight of DHA to ARA was based on data available in 1992 about typical levels of these two fatty acids in human milk samples from women in North America. However, those data may reflect a high intake of omega-6 PUFAs in the North American diet at that time and may not be optimal for long-term health. ARA and its metabolites are proinflammatory lipidic mediators; they may play a role in allergic and asthmatic responses. The United States is experiencing trends of increasing prevalence of asthma and allergy beginning in childhood. High dietary levels of omega-6 PUFAs are also associated with a range of chronic diseases including coronary artery disease, hypertension, and immune/inflammatory disorders. Worldwide, women on healthier diets than the typical American diet have a higher ratio of DHA to ARA in their milk than this 1:2 ratio. From these considerations, Dr. Birch concluded that the recommended ratio of omega-3 to omega-6 PUFAs for dietary supplementation needs to be reassessed.

Infant formula supplementation has been studied for 20 years, so there are cohorts of children now in their teens who participated in the studies of feeding infants fortified or unfortified formula. Continuing to follow the general health of these subjects could provide insights into how infant and early childhood nutrition, including DHA intake, continues to influence cardiovascular health, respiratory health, body composition (e.g., obesity trends), and immune function. Finally, given that the existing studies have not found a plateau in DHA accumulation in the brain and retina out to 12 months after birth, another area for research is whether DHA supplementation should be continued through the second year of life.

Post-Presentation Discussion

In response to a question from Dr. Emily Chew, Dr. Birch said that intravenous feeding of premature infants to provide total parenteral nutrition has recently begun to include DHA supplementation. The prac-

tice began in Europe; within the past year or so, it has begun in some U.S. urban hospitals. In response to a question from Dr. Sheila West, Dr. Birch said that her group's studies of DHA supplementation have found no gender differences, even though about 2,000 children have been studied thus far. Dr. Caroline Klaver asked about data on outcomes and DHA intake for children older than 1 year and whether later DHA supplementation could make up for a prior deficit during that period. Dr. Birch said that children between 1 and 4 years who have low DHA intake have outcomes similar to those of infants on unfortified formula. Whether supplementation can make up for an earlier deficit is not known. A difficulty with dietary supplementation of omega-3 PUFAs in young children is developing a supplement that they will eat.

Dr. Nicolas Bazán endorsed the point made by Dr. Birch that the typical American diet is deficient in essential nutrients such as DHA and other omega-3 PUFAs. This deficiency could have substantial unexpected future consequences, analogous to potential consequences of widespread refractive surgery as discussed during and after Dr. West's presentation. DHA is an essential component of all neural synapses and of the specialized photoreceptor neurons of the retina. Dr. Bazán's comment led to continued discussion among the symposium participants on cultural differences as a factor in infant and childhood nutrition. Parental influence on mental and visual development is also important; as an example, Dr. Birch noted that the breastfed groups in her studies always have brain and visual outcomes several points better than the formula-fed groups.

Roles of DHA and Its Bioactive Derivatives in Chronic Eye Diseases

Dr. Nicolas Bazán

Dr. Bazán and his colleagues have been seeking to understand the physiological significance of omega-3 PUFAs, including their role in the photophysiology of vision. For many years, the prevailing view was that

these fatty acids were mainly important for their influence on membrane fluidity. An initial finding by Dr. Bazán is that DHA is released when cells of the brain and retina are activated—for example, when they are stressed by a decrease in blood supply (ischemia) [99, 100, 101]. In 1984 Dr. Bazán's group hypothesized that the DHA released from membrane phospholipids reflects the initiation of a pathway for synthesis of bioactive derivatives; they coined the term "docosanoids" for these derivatives [102, 103]. His presentation covered a number of topics on the biochemical mechanisms related to cell protection in which DHA docosanoids play specific roles (table 5).

Dr. Bazán's work with colleagues and collaborators has established that one docosanoid in particular, a stereospecific dihydroxylated derivative of DHA called neuroprotectin D1 (NPD1) acts as a cell signaling messenger[6] [104, 105]. In many of the mechanisms discussed by Dr. Bazán, NPD1 protects neural cells such as the eye's photoreceptor and retinal pigment epithelium (RPE) cells from apoptosis in response to oxidative damage. He described the current direction of the work in his laboratory, by analogy with efforts to understand the genetic code in the human genome, as *opening the code of the DHA lipidome*.

Omega-3 Fatty Acids in Corneal Health

Dr. Bazán supplemented Dr. Sheila West's presentation on dry eye syndrome earlier in the symposium by discussing a mechanism by which omega-3 fatty acids may be involved in dry eye. A lipid layer, which protects the outer surface of the cornea and over which the aqueous tear film spreads, consists of lipids secreted by the meibomian glands, which are located in the eyelid. These sebaceous glands are under neuronal, hormonal, and vascular control, and the rate of lipid secretion tends to decrease as sex hormone levels decrease. Among the factors that can alter the lipid composition of the tear film and result in dry eye are age,

[6]The name "neuroprotectin D1" was chosen because of its neuroprotective bioactivity during oxidative stress; its potent ability to inactivate pro-apoptotic and pro-inflammatory signaling; and its status as the first identified neuroprotective messenger derived from DHA.

Table 5. Key Polyunsaturated Fatty Acids and Some Bioactive Derivatives

Precursor fatty acid/ bioactive derivative	Major role
ARA	Important omega-6 fatty acid present in most animal fats; derivatives are *eicosanoids*
Prostaglandins	Perform a variety of hormone-like actions (e.g., blood pressure control, smooth muscle control); promote inflammation
Leukotrienes	Participate in allergic responses (e.g., anaphylaxis)
Lipoxins	Short-lived anti-inflammatory mediators whose appearance typically signals the resolution of inflammation
DHA	A major omega-3 fatty acid in retina, brain, and sperm phospholipids; derivatives are *docosanoids*
Neuroprotectin D1	Inhibits inflammation; promotes neuron survival (neuroprotection)
Resolvins	Inflammation inhibitors derived from DHA or eicosapentaenoic acid (another omega-3 fatty acid) via the COX-2 pathway

gender, diet, systemic medications, environmental conditions, and even wearing contact lenses.

After the work in the 1980s on topical treatment of dry eye syndrome with evening primrose oil (described by Dr. West in her presentation, see p. 53, Oxholm et al. reported that, among 41 Danish patients with Sjogren's syndrome, those with higher serum levels of dietary omega-3 PUFAs had less-severe dry eye symptoms than patients with lower levels of omega-3 PUFAs [106]. Anecdotal results from 116 dry eye patients who took flaxseed oil (a source of omega-3 PUFAs) as a dietary supplement were that 98 percent reported an improvement in their symptoms [107]. As Dr. West had presented earlier, the Women's Health Study included a self-reported dietary questionnaire study of the

association between omega-3 and omega-6 PUFAs in the diet and self-reports of dry eye symptoms. This study indicated that higher dietary intake of omega-3 PUFAs (like DHA) but not omega-6 PUFAs (like ARA) was associated with fewer dry eye symptoms (see p. 53 and [19]).

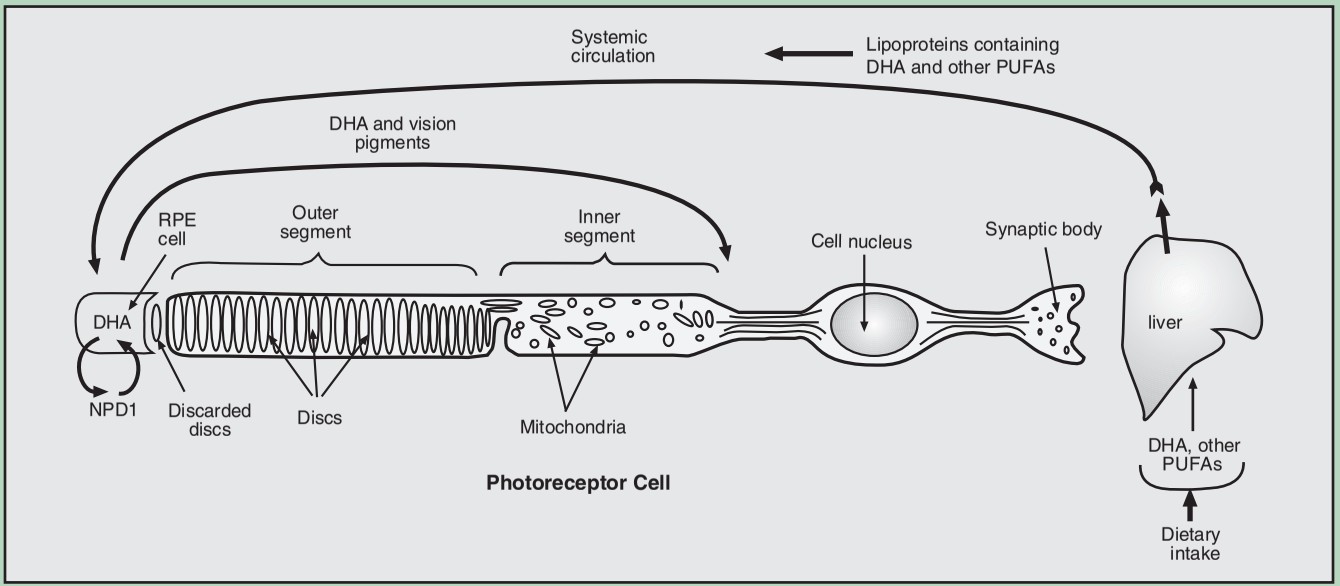
Dr. Bazán said that one of the concerns about refractive surgery is that the epithelial trauma associated with the surgical procedure—as with severe epitheliopathy caused by dry eye syndrome—impairs the normal wound-healing response. Damage to the corneal nerves during refractive surgery also impairs wound healing and can cause epithelial erosions. In collaboration with Dr. Haydee Bazán's group, Dr. Nicolas Bazán has shown that, in a rabbit model for refractive surgery, twice-weekly topical applications of a combination of nerve growth factor and DHA resulted in faster corneal nerve recovery after surgery [108]. This laboratory proof of principle may lead to therapeutic application in treating dry eye and the neural-mediated corneal problems that sometimes arise after refractive surgery.

DHA Role in Photoreceptor Outer Segment Renewal

The outer segment of a photoreceptor cell consists of a stack of photosensitive discs, which contain rhodopsin, a biomolecule that interacts with light (figure 13). Via these photosensitive discs, the cell converts light energy into an electrical signal, which conveys information about the image on the retina to the visual cortex of the brain. To keep this highly energy-intensive process working normally, the photoreceptor cells constantly renew the outer segment by shedding the oldest discs out at the tip and adding new discs at the base of the outer segment. Photoreceptor cells are aligned in parallel, with their outer segments juxtaposed to the RPE cell layer behind them in the multilayered retina. As discs are shed from the tip of a photoreceptor outer segment, an RPE cell aligned with that tip captures the shed disc packets, ingests them, and breaks them down into simpler chemical components in a process of *phagocytosis*.

Photoreceptor outer segments have the highest DHA content of any cell in the body, Dr. Bazán noted, and by working with their associated RPE cells, the photoreceptor cells recycle and thus retain their DHA through multiple cycles of outer segment renewal. This cycle for con-

Figure 13. RPE Cell Phagocytosis of Outer Segment Discs and Resupply of DHA and Other PUFAs via the Systemic Circulation



Source: Adapted from Dr. Nicolas Bazán, presentation to the Fifth Drabkin Symposium, June 2007.

serving DHA is so effective that, together with DHA storage in the liver, prolonged dietary deprivation of omega-3 PUFAs is required to reduce the DHA content in the retina to the point that functional impairments result.

Nevertheless, DHA supplementation may be necessary at specific times and under special conditions. As Dr. Birch's presentation highlighted so well, ensuring sufficient DHA in the diet of infants and pregnant or nursing women is essential to meeting the needs of the rapidly developing brain and retina from the third trimester of fetal development through at least the second year of childhood. Maturation of retinal function and visual acuity are affected when DHA supply is constrained, as is overall neurological performance. Serum DHA decreases in various forms of human RP [109, 110], in Usher's syndrome [111], and in animal models of inherited RP. (See Dr. van Veen's presentation, p. 93, for discussion of animal models.) One such model, a rodent model with a rhodopsin mutation homologous to mutations in some forms of human RP, also had decreased levels of DHA in the photoreceptor cells [112]. All of the mechanisms linking serum DHA, retinal levels of DHA, and RP or RP-like changes in photoreceptor cells are not yet known, Dr. Bazán said, but part of the larger story involves the docosanoid NPD1.

NPD1 as a Cellular Messenger for Protection of RPE and Photoreceptor Cells

NPD1 is a stereospecific lipid produced from DHA when RPE cells undergo oxidative stress [105, 113]. The work in Dr. Bazán's laboratory has not only established the synthesis route for NPD1 from DHA released from membrane phospholipids but also the potency of NPD1 in inhibiting apoptosis and inflammation responses to oxidative stress experienced by both RPE and photoreceptor cells [113, 114, 115]. NPD1 synthesis increased in cultured RPE-derived cells (ARPE-19 cell line and in human RPE cells in primary culture) that were oxidatively stressed while also being supplied with photoreceptor outer segments for phagocytosis. Phagocytosis of outer segment discs was found to provide a rich supply of DHA as the precursor to NPD1. When supplied with outer segments, RPE cell apoptosis was less than half the rate without the pres-

ence of outer segments [114]. In work not yet published, Dr. Bazán's group has shown that this protective effect of outer segment phagocytosis, with concomitant release of DHA and production of NPD1, is even more pronounced in primary human RPE cells prepared from donor human eyes.

Neurotrophins are proteins that act as neuroprotectants (neuron-survival agents) for many cell types. They are also highly potent growth factors, modulating the development, differentiation, and maintenance of structure and function in different types of neurons. NPD1 synthesis was promoted by the presence of the neurotrophin pigment epithelium–derived factor (PEDF) and other factors when oxidatively stressed RPE cells were ingesting outer segment discs [115]. The combination of PEDF and free DHA in the culture medium enhanced RPE cell survival more than either PEDF or DHA alone. Furthermore, the PEDF was much more effective in enhancing NPD1 synthesis in the RPE cell and release to the extracellular medium when it was added to the side of the RPE cells that normally would face the photoreceptor cells (the apical side) than when the PEDF was added to the side of the RPE layer that normally faces away from the photoreceptors (basolateral side). Enhanced production of NPD1 thus appears to be at least part of the mechanism by which neurotrophins like PEDF promote the survival of RPE and photoreceptor cells under stressed conditions that otherwise lead to apoptosis [115]. Thus, NPD1 appears to be a cell signaling messenger promoting cell functional integration, and neurotrophins such as PEDF seem to be important NPD1 activators.

NPD1 also inhibits inflammatory responses, including COX-2 (an enzyme responsible for synthesis of prostanoids that act as major mediators of inflammation) induced by interleukin 1ß [105] and 15-lipoxygenase-1. Dr. Bazán described studies of stereospecific binding of NPD1 that indicate there is a single binding-site receptor for this messenger molecule in RPE cells. The molecular biology of this binding site is under investigation.

Opening the DHA Lipidome Code

Dr. Bazán described the approach that he and his colleagues and collaborators have taken in studying DHA and NPD1 as "functional lipidomics." For years it has been known that DHA was involved in a broad

array of neural system functions: brain development, memory formation, synaptic membrane function, photoreceptor biogenesis and function, and neuroprotection. The studies in functional lipidomics are beginning to show how these functions are carried out at the level of biochemical mechanisms.

Dr. Bazán contrasted the bioactivities of derivatives of ARA, such as prostaglandins, leukotrienes, and lipoxins (which are collectively called "eicosanoids"), with the role of docosanoids such as NPD1. Docosanoid signaling appears to have important positive roles in modulating inflammation, neuroprotection, neurorepair, and neuroregeneration. Dr. Bazán sees this as an area where many concepts for understanding the progression or inhibition of chronic degenerative disease begin to intersect, such as the role of dietary omega-3 PUFAs in a generally healthier diet, the role of neurotrophins, modulation of the inflammatory response, regulation of gene expression, and influence on factors that promote or threaten cell survival through their impact on cell integrity. For example, PEDF is known to be anti-angiogenic, and there may be potential for PEDF and NPD1 to synergistically inhibit the new blood vessel formation (angiogenesis, neovascularization) that is often the proximate cause of severe vision loss and blindness in retinal degenerative diseases such as AMD and DR.

Dr. Bazán concluded his presentation with the following implications for chronic diseases of the eye. DHA-oxygenation pathways lead to the synthesis of novel retinal and corneal nerve messengers (such as NPD1). These messengers mediate neuroprotective signaling responses to oxidative stress, cell injury and damage, and neurodegeneration. They therefore present *potential targets* for therapeutic interventions that involve neuroprotective or even neuroreparative mechanisms. Among the eye diseases for which such interventions may be effective are AMD and other retinal degenerations (RP and proliferative diabetic retinopathy), ischemic retinal diseases, corneal nerve regeneration and dry eye syndrome, and other neurodegenerative diseases including possibly glaucoma.

Post-Presentation Discussion

Dr. Allen Taylor asked how one might pursue advantageous applications of NPD1. For example, are the biological roles (e.g., as a messenger with

a specific binding site) more important than possible chemical roles? How important is its stereospecificity to potential therapeutic applications for combating oxidative stress or modulating cellular functions and apoptotic progression? Dr. Bazán agreed that these are important questions and noted that his group is currently involved in several collaborations with chemists and molecular biologists at other institutions to address some of the issues raised in Dr. Taylor's questions. One approach is to investigate analogues that retain some of the specificity of action of NPD1. Another approach is to determine overall physiological effects such as by infusing NPD1 directly into models of ischemic damage. Dr. Taylor and Dr. Bazán agreed that more work that will be needed to understand how the activity of NPD1 is linked with specific cellular processes such as lysosomal functioning within RPE cells. In response to a question from Dr. Gerald Chader, Dr. Bazán summarized what is known about the effects of NPD1 on gene expression in RPE cells, including its inhibition of proinflammatory COX-2 expression.

Dr. Birch commented that, if the DHA supply is inadequate during the period of rapid neural development, the structural effects on the brain and retina are not reversed by later DHA supplementation. She asked whether NPD1 production was subject to the same constraints, or if it could be successfully increased (up-regulated) even after an early period of DHA insufficiency. Dr. Bazán replied that the answer to that is not yet known. He described a collaboration that had begun on that question, which had been disrupted when Hurricane Katrina damaged his laboratory in New Orleans.

Alternative Therapies: What Works and What Doesn't

Dr. Sayoko Moroi

Dr. Moroi's presentation began with reasons why the clinical and research communities in ophthalmology, as well as public health policy makers and administrators, should care about complementary and alternative medicine (CAM) for chronic eye diseases. She illustrated

the range of CAM therapies that fall under the definition established by the National Center for Complementary and Alternative Medicine (NCCAM). She described her review of the medical literature on the scientific evidence relevant to CAM supplements promoted for eye health and discussed how health care providers can make "best value" judgments in advising patients on CAM. Her concluding topic was the potential implications for present and future incorporation of CAM in medically responsible clinical management.

Why Should We Care about Alternative Therapies?

To illustrate patients' interest in CAM and clinician responsibilities in advising about CAM options, Dr. Moroi, who is a glaucoma specialist, started with some personal anecdotes. During a recent clinical visit with Dr. Moroi, a 53-year-old female patient who has high myopia and optic nerve damage from glaucoma asked, "Will ginkgo or memantine help me?"[7] On the flight to attend the symposium, Dr. Moroi saw an advertisement for a human growth hormone supplement in the airline's inflight magazine that claimed this supplement "is known to reverse" ailments ranging from hemorrhoids and autoimmune disease to macular degeneration, cataract, diabetic neuropathy, and high blood pressure. These two isolated incidents reflect the growing knowledge about and use of CAM by Americans, as well as commercial interest in expanding markets for CAM products and services. Moving from anecdotes to national statistics, Dr. Moroi cited a study that estimated Americans spent $27 billion in 1997 on CAM therapies and professional services. This amount includes homeopathic professional services as well as over-the-counter medications [16]. Growth in this market continues to be rapid, and by 2000, the amount spent in the United States on herbal supplements alone was estimated at $1.4 billion [17].

[7]Memantine is the generic name for a bioactive compound (1-amino-3,5-dimethyl-adamantane) that acts on the glutamatergic system by blocking NMDA glutamate receptors. It has been approved for treatment of moderate to severe Alzheimer's disease, but controversy continues about its effectiveness, particularly given its cost. According to the Wikipedia entry for memantine, it is currently being tested as a medication for glaucoma and a range of other diseases.

In spite of the extensive and growing use of CAM, there are significant medical concerns about both the safety and efficacy of individual CAM options. On the safety side, users are risking adverse effects including toxicity, often from contaminants of the formulation rather than the purported active ingredient, and potential adverse interactions of the CAM product with their prescribed medications or other CAM products they are using. Despite these risks, Americans are using CAM products in greater amounts each year because of their hope or belief, often stimulated by advertising claims, that a product will help their condition. Efficacy is therefore a central issue.

National statistics on CAM use specifically for chronic eye disease are sparse. Dr. Moroi cited the advertising claims for a human growth hormone product to illustrate that many of these products are promoted and used for a range of ailments. Estimates of national sales of such products do not provide data specifically on their use for eye diseases. However, Dr. Moroi reported on two studies of CAM use by patients of ophthalmology medical practices or medical centers/clinics, which show that patients with eye diseases are indeed using these products. One of these studies was the 2002 study, described in section 1 (p. 43) of CAM use by patients of two large urban glaucoma practices [18]. The second was a cross-sectional survey by Dr. Moroi and her colleagues of the daily use of vitamins and herbal supplements by 397 patients at the University of Michigan Comprehensive Ophthalmology Clinics [116].

In the University of Michigan survey, 58 percent of the respondents (230 patients) took a daily vitamin supplement, with 46 percent taking a multivitamin supplement. Eight percent (32 patients) used an herbal product daily. Twenty-six percent (103 patients) had learned about the benefits of vitamins from their primary care physician, but only 2 percent had learned about vitamins from their ophthalmologist. The survey was conducted around the time of the results on the AREDS trial of antioxidant and zinc supplements. Only 2 percent of these patients had learned about herbal CAM products from their primary care physician, and less than 1 percent had heard about them from an ophthalmologist. The average monthly expenditure on vitamins by these patients was $15.74, while users of herbal products spent an average of $15.35 [116].

What Is Included in CAM?

To provide a working definition of alternative therapies for her review of the medical literature, Dr. Moroi used the concepts of *conventional medicine*, *complementary medicine*, and *alternative medicine* as stated by the NCCAM [9]:

- *Conventional medicine* is medicine as practiced by holders of M.D. (medical doctor) or D.O. (doctor of osteopathy) degrees and by their allied health professionals, such as physical therapists, psychologists, and registered nurses.
- *Complementary medicine* is used *together with* conventional medicine. An example of a complementary therapy is using aromatherapy to help lessen a patient's discomfort following surgery.
- *Alternative medicine* is used *in place of* conventional medicine. An example of an alternative therapy is using a special diet to treat cancer instead of undergoing surgery, radiation, or chemotherapy that has been recommended by a conventional doctor.

With these definitions, the NCCAM distinguishes complementary medicine from alternative medicine based on whether a treatment is used to supplement conventional treatment or replace it. Taking the two nonconventional categories together, the NCCAM characterizes CAM generally as "a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine."

There are five domains of CAM defined by NCCAM [9]:

- Whole medical systems (e.g., homeopathic and naturopathic medicine, traditional Chinese medicine, Ayurveda)
- Mind-body medicine (e.g., prayer, meditation, mental healing, art and music therapies)
- Biologically based practices (e.g., dietary supplements, herbal products, and vitamins when used in ways not sanctioned by conventional medicine)
- Manipulative and body-based practices (e.g., chiropractic and osteopathic manipulation, massage)

- Energy medicine (e.g., qi gong, Reiki, Therapeutic Touch, and electromagnetic field therapies)

Dietary supplements fall under the NCCAM domain of biologically based practices. A dietary supplement is a product intended to supplement the diet by increasing total dietary intake of one or more vitamins, minerals, herbs or botanicals, amino acids, other dietary substances, or combinations of these. Dietary supplements are intended for ingestion in pill, capsule, tablet, or liquid form and are not represented for use as a food in the conventional sense or as the sole item of a meal or diet. Dr. Moroi noted a number of myths about dietary supplements, which are commonly believed by those who use or promote them. One myth is that supplements are not drugs in the medical sense. Another myth is that they are safer than pharmaceuticals.

The FDA currently has only a limited role in regulating dietary supplements. The 1958 Food Additive Amendment to the Federal Food, Drug, and Cosmetic Act required that the FDA evaluate the safety of all *new* ingredients, including those used in dietary supplements. The 1994 Dietary Supplement Health and Education Act, whose stated purpose was to help constrain the growth in health care spending while improving the health status of Americans, clarified the 1958 position by explicitly stating that dietary supplements are not "drugs" with respect to drug oversight by the FDA. According to the FDA:

> By law [the 1994 Dietary Supplement Health and Education Act], the manufacturer is responsible for ensuring that its dietary supplement products are safe before they are marketed. Unlike drug products that must be proven safe and effective for their intended use before marketing, there are no provisions in the law for FDA to "approve" dietary supplements for safety or effectiveness before they reach the consumer. Also unlike drug products, manufacturers and distributors of dietary supplements are not currently required by law to record, investigate or forward to FDA any reports they receive of injuries or illnesses that may be related to the use of their products [118].

For CAM products already on the market, the FDA has the burden of proving that a product is unsafe before ordering that it be removed from

the market. The 1994 act also established labeling requirements, including the required disclaimer, "Statements contained herein have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure or prevent any disease." If a dietary supplement's label claims that it can diagnose, treat, cure, or prevent a disease, then it is an illegal drug because it has not been approved by the FDA. The symposium participants discussed the discrepancy between a commonsense reading of this labeling requirement and the advertising claims for supplements, such as the above example of a human growth hormone product that Dr. Moroi had found prominently advertised in consumer-oriented magazines.

Dr. Moroi suggested that certain "off-label" uses of conventional therapies be included in the scope of alternative therapies. Her examples included the following treatments for eye diseases:

- Mitomycin C as an antibiotic in filtration surgeries for glaucoma (mitomycin C is approved as an antitumor antibiotic agent for use in chemotherapy)
- Memantine for neuroprotection in glaucoma patients (memantine is approved for treatment of moderate to severe Alzheimer's disease)
- Rheopheresis®, a proprietary form of membrane differential filtration, or apheresis, for dry AMD (apheresis is used to treat vascular diseases; Rheopheresis removes substances such as low-density lipoprotein cholesterol, fibrinogen, and lipoprotein A, which supposedly accumulate in the blood of some AMD patients) [117]

If a medical practitioner wants to suggest or recommend a CAM dietary supplement to a patient, Dr. Moroi noted that there are additional considerations that are typically not necessary when prescribing a conventional treatment. First, there are large variations in ingredients and in purity, potency, and sources of ingredients between CAM supplement brands, between lots of the same brand, and even within the same bottle. Second, supplements can interfere with biomedical tests in unexpected ways: one source reports that 15 to 20 percent of nutritional supplements sampled contained nonlabeled substances that can, for exam-

ple, produce positive results in tests for controlled substances (e.g., sports or employment illicit drug tests) [119]. Dr. Moroi recommended that physicians instruct their patients to look for products with the United States Pharmocopoeia (USP) seal. The USP is an independent, science-based public health organization that, among other activities, establishes standards for quality and purity of supplements.

The Scientific Literature on Selected Alternative Therapies for Eye Disease

For the Fifth Drabkin Symposium, Dr. Moroi had been asked to review the scientific literature supporting or refuting claims for three specific substances often promoted for purported eye-health benefits. These three are leaf extracts of the ginkgo biloba tree, berry extracts of bilberry (*Vaccinium myrtillus*), and berry extracts of wolfberry (*Lycium barbarum*).[8]

Although the composition of ginkgo biloba leaf extracts is complex, the principal active compounds are presumed to be flavonone glycosides (22–27 percent by weight) and terpene lactones (5–7 percent by weight). The glycosides may act as free-radical scavengers. The lactones may inhibit platelet-activating factor. Other claimed effects relevant to major eye diseases and eye health in general include inhibition of chemically induced apoptosis, inhibition of nitric oxide, increases in (up-regulation of) endothelium-derived relaxation factor, and enhanced neurotransmission (perhaps via catecholamine and indolamine pathways).

Based on the clinical trial evidence cited on the National Library of Medicine's MedlinePlus website for patient information on drugs and supplements,[9] there is strong evidence for efficacy of ginkgo biloba in peripheral vascular disease and some forms of dementia. There is good evidence for efficacy with cerebrovascular insufficiency. However, the evidence for glaucoma and macular degeneration is unclear. The NCCAM is sponsoring or has sponsored a number of clinical trials of ginkgo biloba, including trials for asthma, cognition outcomes, drug

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[8]For general information on active compounds, mechanisms of action, and claimed effects, Dr. Moroi used references 120, 121, 122, and 123.

[9]www. nlm. nih. gov/medlineplus/druginfo/natural/patient-ginkgo. html. Accessed by Dr. Moroi on June 23, 2007.

interaction, peripheral vascular disease, sexual dysfunction, and multiple sclerosis.

Among the precautions relevant to this herbal supplement are serious neurological and allergy reactions from ingesting the raw fruit or seeds of the ginkgo, which contain a toxin. Ginkgo biloba should not be used if patients are taking an antiplatelet drug or a blood-thinning drug such as warfarin. Given its antiplatelet activity, patients should discontinue use of ginkgo biloba 7–14 days prior to surgery, to decrease the risk of bleeding.

The folklore about bilberry's efficacy in improving night vision originated in a military disinformation ploy by the British during World War II. The story that Royal Air Force pilots were eating bilberry preserves to improve their vision was disseminated to mask the introduction of radar for air defense and detection of surfaced submarines. Potentially bioactive compounds in bilberry include tannins, flavonol glycosides, and anthocyanosides in the berries. Compounds in the leaves are claimed to have anti-proinflammatory and hypoglycemic properties. A review of placebo-controlled clinical trials reported efficacy for acute diarrhea, oral mucosal membrane irritation, and venous insufficiency, but no effect in improving night vision [124]. Because of its potential antiplatelet effect, patients taking bilberry should stop using it prior to surgery.

Wolfberry was included in the first known Chinese pharmocopoeia, which included 365 medicines and is attributed to the mythological emperor Shen Nung (Divine Husbandman), who according to legend lived in the 28th century BCE. Among the bioactive compounds in the berries are calcium, potassium, iron, zinc, selenium, riboflavin, vitamin C, beta-carotene, zeaxanthin, and polysaccharides. Reported uses for wolfberry include enhancing the immune system, improving eyesight, protecting the liver, boosting sperm production, and enhancing circulation. A potential mechanism of action is via the antioxidant compounds in the berries. A 2007 study reported that wolfberry promoted survival of retinal ganglion cells in a rat model of elevated IOP and glaucoma. However, the elevated IOP of animals eating 250–280 g per day of the red berries was not altered [125].

The second CAM topic that Dr. Moroi had been asked to review was visual neuroprosthesis. The goal of creating an artificial form of vision as a prosthesis in cases of retinal degeneration traces back to attempts in the 18th century to use direct electrical stimulation of blind eyes. In the 1920s, direct electrical stimulation of the visual cortex of the brain was tried. The current approaches—using cortical implants, optic nerve electrodes, and subretinal or epiretinal prostheses—incorporate improvements in surgical techniques and technology advances in areas such as electronic miniaturization, electrode design, and new biocompatible materials [126]. At least one clinical trial of an epiretinal prosthetic device is in progress.

One study has reported that subretinal implants appeared to have a neurotrophic effect on photoreceptors and perhaps other retinal cells in patients who have RP. A possible mechanism is stimulation of the surviving rod and cone photoreceptor cells [127]. In Royal College Surgeon rats, a rodent model for RP, there was histologic and electroretinogram evidence that photoreceptors were preserved. The apoptosis pathway appears to be altered, compared with controls [128, 129].

To illustrate CAM-related issues with certain off-label uses of conventional treatments, Dr. Moroi discussed Rheopheresis, a proprietary form of apheresis that has been promoted for treating advanced AMD. In general, "apheresis" refers to withdrawal of blood from a donor or patient, removal of one or more components (for example, plasma, blood platelets, white blood cells, or, in Rheopheresis, high molecular weight proteins) from the blood, and transfusion of the remaining blood components back into the donor or patient. Variants of apheresis are conventional treatments for managing Guillain-Barré syndrome, myasthenia gravis (an autoimmune disease causing progressive weakness and exhaustibility of voluntary muscles), and some forms of hyperlipidemia (excess fat or lipids in the blood). In Rheopheresis, the plasma in the withdrawn venous blood is separated from the blood cells, then filtered to remove high molecular weight proteins. The filtered plasma is remixed with the blood cells and returned to the patient's circulation. The hypothesis supporting the procedure is that

removal of the large proteins increases blood flow by decreasing viscosity, decreasing erythrocyte and thrombocyte aggregation, and improving erythrocyte flexibility.

A multicenter prospective, randomized, placebo-controlled and double-masked phase 3 clinical trial of Rheopheresis was conducted with 216 patients with category 3 or 4 advanced AMD (using the AREDS categories) [130]. Patients received either the apheresis-with-filtering treatment or a sham treatment. The course of treatment was eight treatments over 10 weeks, and outcomes were measured at 3, 6, and 12 months. At 12 months, there was no difference in vision between 104 Rheopheresis-treated subjects and 63 sham-treated subjects. Because a number of subjects had been included who did not meet the original enrollment criteria of the trial, a subgroup analysis was conducted by excluding 37 percent of the Rheopheresis-treated and 29 percent of the sham-treated subjects. In this subgroup, there was statistically significant improvement in the Rheopheresis-treated patients at 1 year, compared with the sham-treated patients. There were 27 adverse events, but only 1.8 percent of the treatments were suspended because of an adverse event. The trial evaluators concluded that the study was at best flawed, in that 37 percent of the treated subjects did not meet inclusion criteria, and at worst there was no evidence of effect. They recommended that, because of the flawed evidence for effect and the occurrence of adverse events (although a small number), Rheopheresis should not be performed for AMD except as part of an approved randomized controlled trial [130]. Dr. Moroi said that an open label clinical study is ongoing for Rheopheresis, using different treatment inclusion criteria and different methods of evaluating outcome. The symposium participants discussed the conditions of the study, the reasonableness of the hypothesis for treatment, and whether any of the outcomes indicated a clinically significant benefit.

The off-label use of memantine for glaucoma, Dr. Moroi said, is based on the hypothesis that cell damage due to glutamate binding to receptors (glutamate excitotoxicity) is a factor in the pathogenesis of glaucoma. A proprietary prospective trial of memantine treatment in patients with open-angle glaucoma has been conducted by Allergan, but

the results had not been made public at the time of this symposium.[10] The participants discussed the outcomes for treatment of Alzheimer's disease that had led to FDA approval of memantine for that condition.

CAM in Clinical Management: Present and Future

Dr. Moroi's closing topic was the present and future status of CAM in clinical management of the chronic, blinding eye diseases on which the Fifth Drabkin Symposium focused. That status is reflected in the NCCAM concept of *integrative* or *integrated medicine* as combining treatments from conventional medicine and CAM for which there is some high-quality evidence of safety and effectiveness [9].

Dr. Moroi suggested the following approach for clinical ophthalmologists in providing their patients who are interested in CAM therapies with best value judgments about them:

- Be sensitive to patients who are curious (and sometimes desperate) about CAM treatments for a blinding and incurable disease.
- As part of taking patients' drug history, ask them about supplements they are taking.
- Health care providers should educate themselves and their patients about both the benefits and risks of CAM options.

At present, good clinical management means using one's best clinical judgment to recommend for or against CAM options with due consideration of both potential benefits (efficacy) and risks (economic costs, side effects, and toxicity). The larger context, which will become increasingly important to practical clinical judgment in the future, is that medical practitioners need to accept and work with the complexity implied by our emerging knowledge of genomics, proteomics, and metabolomics, as well as the interactions of these functional complexes with environmental factors that affect health and disease.

Dr. Moroi sees two major challenges in effectively integrating CAM with conventional treatments. First, the resources are severely limited

[10] A subsequent report from the second phase 3 clinical trial found no significant benefit of memantine on glaucoma progression compared to placebo. www.glaucoma.org/treating/memantine_updat_1.php, accessed April 25, 2008.

for well-designed studies of CAM options on diseases with a major public health impact. The second challenge is in translating the knowledge gained about molecular-level processes and cell biology interactions occurring in both the eye and the entire body into improved diagnostics and therapeutics for preventing and treating eye diseases.

Post-Presentation Discussion

In response to a question on the evidence for beneficial effects of flavonoid compounds, Dr. Moroi repeated the point made by earlier speakers that natural products are typically very complex chemical mixtures, and the compositions of products on the market vary greatly. This led to general discussion of the problems in credibility of the information on ingredients in a variety of nonprescription supplements, including supplements that claim to have specified amounts of the AREDS antioxidants or of lutein/zeaxanthin but in fact have far lower amounts. Another example is the large number of ginkgo biloba and DHA products on the market and the lack of reliable information on their composition, potency, and purity. Dr. Bazán described his testing of DHA supplements, which he found to have up to 20 percent lipid peroxides by weight. Dr. Chew noted that lutein/zeaxanthin supplements with substantial amounts of the active compounds are still quite expensive to manufacture. This led to a consensus among the participants on the need for greater study of the cost versus efficacy of a range of dietary supplements, whether used in conventional treatment or in CAM options.

Alternative Therapies for Glaucoma

Dr. John Hetherington

Dr. Hetherington's presentation was based on his review of the scientific literature on complementary or alternative therapies that are being used for glaucoma, together with his experience in treating glaucoma patients who are either using or inquiring about CAM for glaucoma.

Vitamin Supplementation

The first area Dr. Hetherington discussed was the evidence in the biomedical literature related to use of vitamins in treating glaucoma. Based on this review, he concluded there was no proven value of vitamin supplementation for glaucoma.

He found no evidence for an effect of either vitamin A (retinol) or vitamin B1 (thiamine) on either IOP or visual field. One study of vitamin B12 reported an improvement in visual fields after 9 months, but Dr. Hetherington questioned the results because visual field changes require more extended follow-up than 9 months and this study was poorly controlled [131].

In principle, vitamin C seems a likely candidate for beneficial effect because it is an antioxidant and is normally at higher levels in the aqueous humor than in the body generally. Dr. Hetherington described a study, in which he was involved, that had produced IOP lowering with large doses of vitamin C, but the patients had such severe side effects (diarrhea and dehydration) that the study was terminated. At the optic nerve head in the retina, where the vision loss from glaucoma occurs, free radicals may be one of the oxidative stresses that mediate apoptosis of retinal ganglion cells, and vitamin C could conceivably have a positive effect. Another possibility is that its antioxidant properties may protect cells in the trabecular meshwork from damage that restricts fluid outflow and thus increases IOP.

Vitamin E supplementation produces no change in the IOP of glaucoma patients. An uncontrolled study reported an expansion of the visual field in glaucoma patients who took a vitamin E supplement [132]. One hypothesis is that, because vitamin E inhibits cell proliferation, it may have the side effect of helping to prolong the benefit of filtering surgery.

As part of the Nurses' Health Study and the Health Professionals Follow-up Study, the dietary intake of antioxidants was evaluated, using diet questionnaires, for 474 subjects with confirmed chronic open-angle glaucoma (COAG). A multivariate analysis, which compared the highest and lowest quintiles of cumulative dietary intake, showed no statisti-

cally significant association between antioxidant consumption and risk of COAG [133].

Marijuana and Derivatives as Alternative Treatment for Glaucoma

Dr. Hetherington emphasized that marijuana, which is used by some glaucoma patients as a CAM, contains many components representing more than 400 distinct chemicals identified to date. There has been no proven effect of marijuana use on visual field loss in patients with low-tension glaucoma or COAG. Although smoking or eating marijuana, or intravenous administration of extracted components, lowers IOP by as much as 25 percent, the effect is short-lived—only 3 to 4 hours. Side effects included lower blood pressure but increased pulse, loss of concentration and coordination, and increased risk of emphysema [134–137].

The marijuana chemicals that have received the most interest are the cannabinoids. Several of these or derivatives have been studied or are currently under investigation [134]. Intravenous administration of dexabinol, a synthetic, nonpsychotropic cannabinoid, was found to produce a dose-related lowering of IOP in albino rabbits [136]. Even with these suggestions of potential effect, the use of marijuana or its derivatives cannot currently be recommended because evidence for positive effects on visual field is lacking and because of marijuana's deleterious side effects.

Herbal Dietary Supplements as Glaucoma Therapy

As a general comment on ginkgo biloba, Dr. Hetherington said that many people, including doctors, seem to take it as a dietary supplement "just in case" it has a positive effect on cognitive ability. Doppler studies have shown that it increases ocular blood flow [138], which suggests it might have a positive effect, particularly for patients with low-tension glaucoma. Nevertheless, the published studies all appear inadequate, Dr. Hetherington said. He concluded that, thus far, ginkgo biloba has not been shown to have a significant positive effect on glaucoma.

As Dr. Sayoko Moroi had noted in her presentation, bilberry is promoted for optical nerve health, although evidence for an effect is lacking. In principle, besides the presence of flavonoids as potential antioxidants, bilberry may also inhibit platelet aggregation, which could possibly

be another mechanism of positive effect. However, Dr. Hetherington found no published studies with evidence of an effect on IOP or visual field.

As Dr. Moroi and other symposium participants had discussed in their presentations, a major issue with commercial dietary supplements, including those purporting to provide antioxidants and vitamins, is the credibility of the claims made about the levels and potency of active ingredients. Dr. Hetherington described an instance of an over-the-counter formulation whose label claimed that it contained 20 mg of lutein per dose. When assayed by the National Eye Institute, the product contained less than 0.02 mg of lutein, or less than a thousandth of the claimed amount.

Lifestyle Factors as Glaucoma Therapy

Diet in general has not been shown to have a significant effect on glaucoma, Dr. Hetherington said. He described a study in which he participated that found that alcohol (4 ounces of 40 percent alcohol) decreased IOP substantially but had too many general cognitive and behavioral side effects to be an acceptable therapy.

Meditation techniques have been found to have a possible long-term effect in reducing IOP, although the effect was not statistically significant. Acupuncture produced no change in patients with elevated IOP, although there is one report of a minor visual change.

In contrast to all of the above CAM options, Dr. Hetherington said that the complementary therapy he recommends to all of his patients is regular, moderate exercise. Physical exercise causes a short-term increase in IOP that is followed by a 14 percent decrease after 1 hour. Moderate long-term exercise increases perfusion to the optic nerve and lowers IOP by an average of 20 percent. The IOP reduction lasts as long as 3 months after a regular exercise regimen is discontinued [139].

Post-Presentation Discussion

Dr. Emily Chew asked if glaucoma patients are using lutein to try to treat their glaucoma. Dr. Hetherington replied that a lot of patients seem to use over-the-counter formulations just in case they may help. Dr. Chew suggested that the AREDS data from the follow-up period could be ana-

lyzed now to determine if there were a significant difference, for lutein versus placebo, on IOP or on the important junction of the optic nerve into the retina (as shown by the stereo disc photographs taken during examinations of AREDS patients). Similarly, the AREDS2 results, when they become available, could be analyzed for the same associations. The symposium participants agreed that these analyses could yield useful information on the efficacy of lutein.

Dr. Moroi noted that she has patients with both AMD and glaucoma. She sees enough of a family association with this type of comorbidity that she suspects there may be common genetic factors for the subgroup of patients with both diseases. They usually have high IOP, she said, and there may be some commonality between effects on the extracellular matrix of the macula and restricted aqueous outflow in the front of the eye. The AREDS and AREDS2 data could be analyzed to look at associations for this subgroup. Dr. Rohit Varma added a cautionary comment that controlling for other factors, such as changes in IOP medication, will be important in looking at epidemiologic data from AREDS and other studies. For example, changes in IOP-lowering medication could affect the evaluation of the effect of antioxidants on IOP.

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Appendix A

Symposium Participants and Observers

Co-Chairs

Gerald J. Chader, Ph.D.
Doheny Retina Institute
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Other Attendees

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Robert J. Katt, Ph.D., *Rapporteur and Consulting Technical Writer*
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Chantilly, Virginia

Appendix B

Symposium Agenda

Monday, June 25, 2007

8:15–8:30 am	Welcoming remarks	Dr. Robert White
Anterior Segment chaired by Dr. John Hetherington		
8:30–9:00 am	Corneal diseases	Dr. Sheila West
9:00–9:15 am	Discussion	
9:15–9:45 am	Cataract	Dr. Allan Taylor
9:45–10:00 am	Discussion	
10:00–10:30 am	Break and informal discussion	
Posterior Segment Chaired by Dr. Gerald Chader		
10:30–11:00 am	Diabetic retinopathy	Dr. Rohit Varma
11:00–11:15 am	Discussion	
11:15–11:45 am	AMD: nutrition & environment	Dr. Caroline Klaver
11:45 am–12:00 pm	Discussion	
12:00–1:00 pm	Lunch	
1:00–1:30 pm	AMD: antioxidants	Dr. Emily Chew
1:30–1:45 pm	Discussion	
1:45–2:15 pm	Retinitis pigmentosa	Dr. Theo van Veen
2:15–2:30 pm	Discussion	
Focus Topics Chaired by Dr. Emily Chew		
2:30–3:00 pm	Neonatal nutrition	Dr. Eileen Birch
3:00–3:15 pm	Discussion	
3:15–3:45 pm	Break and informal discussion	
3:45–4:15 pm	Bioactive lipids	Dr. Nicholas Bazán
4:15–4:30 pm	Discussion	

4:30–5:00 pm	Alternative therapies: what works and what doesn't	Dr. Sayoko Moroi
5:00–5:15 pm	Discussion	
5:15–5:30 pm	Introduction to a final report	Dr. Robert White Dr. Robert Katt

Tuesday, June 26, 2008

Reports, Draft Recommendations, and Discussion

8:30–8:50 am	Corneal diseases
8:50–9:10 am	Cataract
9:10–9:30 am	Diabetic retinopathy
9:30–9:50 am	AMD—nutrition and environment
9:50–10:10 am	AMD–antioxidants
10:10–10:30 am	Break and informal discussion
10:30–10:50 am	Retinitis pigmentosa
10:50–11:10 am	Neonatal nutrition
11:10–11:30 am	Bioactive lipids
11:30–11:50 am	Alternative therapies
11:50 am–12:30 pm	General discussion and overarching recommendations
12:30 pm	Closing lunch and departure

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