A CRITICAL APPRAISAL OF THE ETIOLOGY OF ADULT HUMAN LENTICULAR OPACIFICATION AND AN INVESTIGATION INTO THE ROLE OF METABOLIC FACTORS IN ITS PATHOGENESIS

David Meyer

Dissertation presented for the Degree of Doctor of Philosophy at the University of Stellenbosch.

Promoter: Dr DP Parkin

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

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SUMMARY

The eye is that biological instrument which conveys the light of the external world into the inner world of the mind, wherein we receive the miraculous gift of vision. So precious is this gift, that Science must search for ways to keep this portal clear for the flow of light. Indeed, Science is called upon to *"make war upon the bloody tyrant, Time."* (Shakespeare W. Sonnet No. 16). For, in the course of ageing, the lens grows cloudy and cataractous. In this battle between Science and Time, we are fortunate to live in an era in which Science is uncovering the molecular basis for the various obstacles to vision. The question arises, whether or not, the ruinous hand of time can be stayed.

Due to unrelenting, progressive lens opacification, most of the elderly are destined to be subjected to loss of vision and with passage of time, even blindness. Globally the cataract surgery rate is inadequate to keep pace with the ever growing demand on financial and human resources created by the cataract problem. An immense challenge therefore is directed to primary eye care: "Can cataract be prevented or can its onset at least be postponed?"

This laudable ultimate aim can only be achieved once the etiology of cataractogenesis is well understood. This dissertation seeks to examine two previously unrecognized etiological aspects that, if correctly understood and managed, have the potential to achieve preventive ophthalmological goals that may indeed help to stay the 'ruinous hand of time'.

The first aspect deals with the role of lipids and was examined using a study group of dyslipidemic subjects. The first part of the study concluded that dyslipidemic patients develop cortical lens opacities more frequently and at an earlier age than the normal population, and that cortical lens opacities should be regarded as one of the most reliable clinical signs of dyslipidemia. Furthermore, an extremely strong correlation was found to exist between low HDL Cholesterol levels and the development of opacities. Below a HDL-Cholesterol level of 1,5mmol/l, subjects had more than seven-fold higher risk of falling in the lens opacity subgroup than those with HDL-Cholesterol levels above 1,5mmol/l. An equally strong correlation was demonstrated between high (>5) LDL:HDL ratios and the development of lens opacities. Subjects with a LDL:HDL-C ratio below 5 possessed a 2.35 times greater risk of having lenticular opacities than the group with a LDL:HDL-C ratio greater than 5. The prevention or retardation of dyslipidemia associated lens opacities is therefore possible, provided patients with a genetic predisposition are detected early and their blood lipids managed adequately.

The second aspect deals with the relationship between age related cataracts and the acetylation status of the individual. This study compellingly submits that the slow acetylator pheno- and genotype may be regarded as a genetic indicator of risk for age related cataract. The ability accurately to classify a patient genotypically and phenotypically, may henceforth be useful in health counseling since, if an individual is identified as being a slow

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acetylator, additional preventative and precautionary measures may be taken, i.e. the prevention of UV-exposure to the eye and caution with the ingestion of xenobiotics like caffeine, commercial dyes, food preservatives, and drugs. Furthermore, such a finding should be taken into account in the long term therapeutic management of glaucoma, with special regard to carbonic anhydrase inhibitors which are sulphonamide-related drugs and totally dependent on the N-acetyltransferase pathway for metabolism. These drugs may accumulate in the slow acetylator over time and exert toxic effects intra-ocularly, conceivably including cataractogenesis.

The search for genetic and metabolic mechanisms that may contribute to human cataractogenesis should be pursued with great enthusiasm. This endeavour may help Science to achieve its primary objective, ablate the effects of Time and really aid in preventing cataracts in man.

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OPSOMMING

Die wondergawe van visie word vir ons moontlik gemaak deur die oog wat as biologiese instrument die lig van die buitewêreld inlaat na die binnewêreld van die brein. So kosbaar is hierdie gawe dat die Wetenskap deurgaans moet poog om diè poort oop te hou. Inteendeel, die Wetenskap word gemaan deur Shakespeare in sy Sonnet nommer 16 om "oorlog te maak teen die bloeddorstige tiran, Tyd". Soos 'n mens ouer word, word die lens dof en 'n katarak mag vorm. Ten spyte van hierdie stryd tussen 'Wetenskap' en 'Tyd' leef ons in die gelukkige era waarin die Wetenskap meer en meer leer van die verskeie obstruksies tot visie. Die vraag ontstaan of die rinnewerende hand van 'Tyd' gestuit sal kan word.

Vanweë ongenaakbare, progressiewe lensvertroebeling is die meeste bejaardes bestem om aan visie verlies, en met verloop van tyd selfs blindheid, te ly. Die wêreldwye katarakchirurgie tempo is nie voldoende om by te hou by die immergroeiende finansiële en mannekrag eise wat deur die katarak probleem gestel word nie. Daar word dus 'n reuse uitdaging aan primêre oogsorg gestel naamlik: "Kan katarakte nie eerder voorkom of die aanvang daarvan ten minste uitgestel word nie?"

Hierdie prysenswaardige doelwit kan nie bereik word alvorens die etiologie van kataraktogenese goed verstaan word nie. Hierdie tesis ondersoek twee voorheen onerkende etiologiese aspekte wat, indien hulle korrek verstaan en hanteer word, beslis die potensiaal het om die gemelde voorkomende doelwitte te bereik en sekerlik te kan bydrae om die rinnewerende hand van Tyd te stuit.

Die eerste aspek spreek die rol van lipiede aan deur te kyk na 'n studiegroep van dislipidemiese persone. Die eerste deel van die studie kom tot die gevolgtrekking dat dislipidemiese pasiente kortikale lens opasiteite meer dikwels en op 'n vroeër ouderdom ontwikkel as die normale populasie en dat sulke opasiteite beskou moet word as een van die mees betroubare kliniese tekens van dislipidemie. Daar is ook 'n baie sterk korrelasie gevind tussen lae HDL cholesterol vlakke en die voorkoms van opasiteite. Persone in die studie met 'n HDL cholesterol vlak laer as 1,5mmol/l het 'n sewe keer hoër kans gehad om in die lensopasiteit subgroep te val as die met 'n HDL cholesterol vlak laer as 1,5mmol/l. 'n Sterk korrelasie tussen 'n hoë (>5) LDL:HDL verhouding en die voorkoms van lens opasiteite is ook gevind. Persone met 'n LDL:HDL verhouding >5 het 'n 2.35 maal groter risiko gehad om lensopasiteite te hê as die met 'n LDL:HDL verhouding van <5. Die voorkoming of vertraging van dislipiedemie geassosieerde lens opasiteite is dus moontlik, solank persone met 'n genetiese geneigdheid daartoe vroeg ontdek en hulle bloedlipiede voldoende beheer word.

Die tweede deel van die tesis handel oor die verhouding tussen ouderdoms verwante katarakte en die asetilasie status van die individu. Met oortuiging kom hierdie studie tot die gevolgtrekking dat die stadige asetilator feno- en genotipe as 'n genetiese merker vir ouderdoms verwante katarakte beskou moet word. Die vermoë om 'n individu beide genotipies en fenotipies akkuraat te klassifiseer mag voorts bruikbaar wees in gesondheidsraadgewing. Indien 'n individu geïdentifiseer is as 'n stadige asetileerder, kan addisionele voorsorg maatreëls getref word soos bv. die voorkoming van blootstelling van die oog aan UV lig sowel as omsigtigheid

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met die inname van xenobiotika soos kaffeïene, kleurstowwe, voedsel preserveermiddels en geneesmiddels. Hierdie bevinding moet ook in berekening gebring word in die langtermyn terapeutiese hantering van gloukoom. Die koolsuuranhidrase inhibitore, dikwels gebruik in die behandeling van gloukoom, is sulfonamied-agtige middels en dus totaal afhanklik van die N-asetieltransferase pad vir hulle metabolisme. Hierdie middels kan ophoop in die stadige asetileerder en gegewe genoeg tyd, bes moontlik toksiese intra-okulêre effekte tot gevolg hê.

Die soeke na genetiese en metaboliese meganismes wat mag bydra tot menslike kataraktogenese behoort nagestreef te word met groot entoesiasme. Hierdie strewe mag dalk net vir die 'Wetenskap' bystaan om sy primêre mikpunt te bereik, die effek van 'Tyd' te neutraliseer en te help om katarakte werklik te voorkom.

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DEDICATION

To my dear and ever supportive wife Marita and our three wonderful children who all share my passion for Medicine and the Healing Sciences - Deidrè, Riegardt and Adèlle

Remember now thy Creator in the days of thy youth, while the evil days come not, nor the years draw nigh, when thou shalt say, I have no pleasure in them.
While the sun, or the light, or the moon, or the stars, be not darkened, nor the clouds return after the rain…and those that look out of the windows be darkened.
Solomon in Ecclesiastes 12:1-3

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS DISSERTATION

PUBLICATONS

1. **D Meyer**: Oogsorg in Suid-Afrika – op pad na 2000. Continuing Medical Education Journal of MASA. Editorial June 1998. Vol 16. No 6, 491-492.
2. **D Meyer**: Cataract prophylaxis – An impossible dream? Continuing Medical Education Journal of MASA. June 1998. Vol 16. No 6, 544-546.
3. **D Meyer**, P H Liebenberg, F J Maritz, L J Burgess: Cortical Opacities in the young patient – an `indication for a lipogram? Investigative Ophthalmology and Visual Science. March 15, 1999. Vol 40. No 4. 5885. (Published Abstract).
4. **D Meyer,** F J Maritz, P H Liebenberg, D P Parkin, L J Burgess: Cortical Opacities in the young patient - an indication for a lipogram? South African Medical Journal. June 2001,Vol 91, No 6 .520-524
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6. **D Meyer,** P H Liebenberg, FJ Maritz: Low High-Density Lipoprotein (HDL) blood levels as a risk factor for the development of Human Lenticular Opacities. Investigative Ophthalmology and Visual Science. March 15, 2001. Volume 42, No. 4 (Published Abstract)

PRESENTATIONS

1. **D Meyer** Presentation: "Cortical lens opacities - an indication for a lipogram in the young patient". Ophthalmological Society of South Africa Congress, March 1-5, 1998. Victoria Falls, Zimbabwe (co-authors: PH Liebenberg, FJ Maritz, LJ Burgess)
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3. **D Meyer** Presentation:. Abnormal serum lipid levels as a risk factor for the development of human lenticular opacification. South African Society of Cataract and Refractive Surgery (SASCRS) and International Intraocular Implant Society (IIIC) Congress. Feb 18-21 2001. Civic Centre, Cape Town(co-authors: PH Liebenberg, FJ Maritz)
4. **D Meyer**. Poster. Low High-Density Lipoprotein (HDL) blood levels as a risk factor for the development of Human Lenticular Opacities. ARVO Congress, April 29 - May 4, 2001. Fort Lauderdale. USA (co-authors: PH Liebenberg, FJ Maritz)
5. **D Meyer.** Presentation: Cortical lens opacities – an indication for a lipogram and low High-Density Lipoprotein (HDL) blood levels as a risk factor for the development of Human Lenticular Opacities. April 25, 2001. Department of Ophthalmology, Loma Linda University, Loma Linda, California, USA. Chairman: Dr Howard Gimbel (co-authors: PH Liebenberg, FJ Maritz)

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6. **D Meyer.** Presentation: Abnormal serum lipid levels as a risk factor for the development of human lenticular opacification. Indian Intraocular Implant and Refractive Surgery Convention. August 24-26, 2001. Chennai, India (co-authors: PH Liebenberg, FJ Maritz)
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TABLE OF CONTENTS

	Page
DECLARATION	ii
SUMMARY	iii
OPSOMMING	v
DEDICATION	vii
ACKNOWLEDGEMENTS	viii
PUBLICATIONS AND PRESENTATIONS	ix
TABLE OF CONTENTS	xi
PREFACE	1
A. BACKGROUND AND INTRODUCTION	1
B. PURPOSE	2
C. HYPOTHESES	3
D. SUMMARY OF MATERIALS AND TECHNIQUES	3
REFERENCES	4
CHAPTER 1	5
LENS ANATOMY AND PHYSIOLOGY RELATED TO CATARACTOGENESIS	
A. INTRODUCTION	5
B. ANATOMIC ASPECTS OF THE LENS	5
C. ASPECTS OF LENS PHYSIOLOGY AND METABOLISM	9
D. WHAT IS UNIQUE ABOUT THE LENS?	15
REFERENCES	17
CHAPTER 2	20
CATARACT ETIOLOGY – A REVIEW	
A. INTRODUCTION	21
B. ADULT CATARACT	22
C. CONCLUSION	38
REFERENCES	39

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CHAPTER 3 48
DYSLIPIDEMIA AND LIPID PHYSIOLOGY - A BRIEF REVIEW
A. INTRODUCTION 48
B. SYSTEMIC LIPID PHYSIOLOGY 48
C. MOLECULAR BIOCHEMISTRY OF CHOLESTEROL 51
REFERENCES 54

CHAPTER 4 57
CORTICAL LENS OPACITIES IN DYSLIPIDEMIC PATIENTS
ABSTRACT 57
A. INTRODUCTION 58
B. METHODS 58
C. RESULTS 60
D. DISCUSSION 65
E. COMMENTS AND CONCLUSIONS 67
REFERENCES 68

CHAPTER 5 69
SERUM LIPID COMPONENTS AS RISK FACTORS FOR LENS OPACITIES
ABSTRACT 69
A. INTRODUCTION: 70
B. METHODS 70
C. RESULTS 72
D. DISCUSSION AND PATHOGENESIS 77
E. CONCLUSIONS 82
REFERENCES 83

CHAPTER 6 86
CONJUGATE ACETYLATION - A REVIEW
CHAPTER COMPONENTS 86
A. INTRODUCTION 86
B. DETOXIFYING ENZYME SYSTEMS 87
C. BIOCHEMISTRY OF ACETYLATION 89
D. ARYLAMINE N-TRANSFERASE IN OCULAR and OTHER TISSUES 89
E. MOLECULAR GENETICS OF NAT 1 AND NAT 2 90
F. POPULATION GENETICS 92
G. LIFESTYLE, ENVIRONMENTAL FACTORS AND DISEASE ASSOCIATIONS WITH N-ACETYLTRANSFERASE 93
REFERENCES 96

Stellenbosch University http://scholar.sun.ac.za/

CHAPTER 7 **102**

AGE RELATED CATARACTS AND ACETYLATION STATUS - A POPULATION STUDY

ABSTRACT 102

A. INTRODUCTION 103

B. MATERIALS AND METHODS 104

C. RESULTS 107

D. DISCUSSION AND PATHOGENESIS 119

E. CONCLUSION 126

REFERENCES 127

APPENDIX **132**

138

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PREFACE

A. BACKGROUND AND INTRODUCTION

In this thesis data will be presented that promote our understanding of the etiology of human lenticular opacification and cataractogenesis. The etiological role of two specific metabolic processes, hitherto not fully recognised as involved in cataractogenesis, will be examined. These are ***lipid metabolism*** as manifested in dyslipidemic subjects and ***conjugative acetylation*** as manifested in a well defined population. It will be shown that cortical lens opacities represent the most reliable clinical sign of dyslipidemia and that low levels of high density lipoprotein (HDL) cholesterol strongly correlate with the development of adult lens opacification. Furthermore, it will be shown that conjugative acetylation as a detoxifying metabolic process is strongly suspected as implicated in human cataractogenesis. Data will be presented that significantly links the slow acetylator pheno-and genotype to adult onset age-related cataract in humans.

Blindness is a major health problem worldwide and most of the blinded reside in the developing countries. Blindness places a significant burden on the community especially in terms of the loss of productivity, financing costs for rehabilitation, civil pensions and human suffering. It is equally true that prevention and reversal of blindness can lead to enormous savings to a country - both financially and in terms of human potential.

Cataracts are internationally regarded as the main cause of reversible blindness. In the Republic of South Africa the estimated cataract blind population is in the region of 155 000[1].

The problem of cataract blindness can be approached from three angles:

1. **SURGERY** – At the present time cataract surgery performed with modern microsurgical techniques and intra ocular lens (IOL) implantations is widely recognized as one of the most cost-effective surgical procedures in the whole of medicine. The availability of this service for all is however limited by the high costs of consumables and skilled manpower shortages.

2. **DRUGS** - The notion of developing anti-cataract drugs is tempting and is being researched actively. To date no such a drug has however been successfully tested[2].

3. **PREVENTION** - The identification and elimination of risk factors for cataractogenesis may therefore prove to be a perfectly sound approach to this challenging problem. *It is precisely on this aspect that this thesis will focus.*

Dr Bjorn Thylefors, Former Director of the WHO Program for the Prevention of Blindness and Deafness and currently involved in the Vision 20/20 Right to Sight campaign, during his Keynote Address and Award at the Association for Research in Vision and Ophthalmology (ARVO) meeting in Fort Lauderdale, Florida, USA on April 29, 2001 stated that "Despite progress made in delivery of surgery, the future lies in the prevention of cataract." and that "More research in the prevention of blindness is needed". This vision was clearly shared by the Department of National Health of South Africa when the health mission statement for the country was

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formulated in its policy document *Health Sector Strategic Framework (1999-2004)* as follows: "Our Mission is ... improving access to health care for all and ... especially *PREVENTIVE AND PROMOTIVE* health[3]. In the light of this formidable challenge a critical analysis of the etiological factors in human cataract formation is long overdue particularly since preventive and promotive medicine has become our national goal.

B PURPOSE OF THIS STUDY

This study will focus on two aspects of the above challenge:

Firstly to identify and critically analyse the recognised and previously reported etiological factors contributing to human lenticular opacification and;

Secondly to examine the etiological role of two specific metabolic factors previously not recognised as cataractogenic factors:

1. Dyslipidemia – a metabolic abnormality in the disposition of **endogenous** lipids;
2. Unfavourable Acetylator characteristics compromising disposition of a wide variey of nitrogenous and potentially toxic, **exogenous** molecules.

Why these two metabolic processes?

1. ***Cholesterol*** is an important constituent of the lens and changes in the composition and contents of the lens sterols have been associated with cataract formation[4]. The lens fibre cell membrane is unique because it contains the highest relative concentration of cholesterol of all membranes[5]. Typically the cells of animals contain 0.5-1.0 moles of cholesterol for each molecule of phospholipid[6]. Although the cholesterol to phospholipid molar ratio in membranes of human lenses vary, it may reach ratios as high as 4:1, being the highest in nuclear membranes[4]. It is believed that cholesterol is important for the maintenance of fluidity between the cortical and nuclear membranes in the lens and that it antagonizes the binding of crystalline proteins to these membranes. It has also been estimated that the cortex of the adult lens is replaced with new growth every 5 years[5]. Cells obtain their cholesterol by *de novo* synthesis or via uptake from circulating lipoproteins. Since the lens grows continuously throughout the life of an individual by terminal differentiation of epithelial cells, a constant need exists for cholesterol and phospholipids. The role of dyslipidemia in the causation of cataract is not immediately evident and needs to be addressed. In particular, it is important to establish the contribution to lenticular opacification of both the duration of dyslipidemia and the impact of the different elements constituting the dyslipidemic profile.
2. ***Conjugative acetylation*** is an important metabolic processing pathway for a large group of chemical substances, many possessing toxic potential to the body. Two separate enzyme systems mediating this detoxification process are well known i.e. the N-Acetyltransferase 1 (NAT 1) and the N-Acetyltransferase 2 (NAT 2) systems. The NAT 2 locus is highly polymorphic in man and several gene alleles coding for this enzyme have been identified[7]. In any given population genotypically there exists three subgroups:

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homozygotic fast (FF), heterozygotic fast (better known as 'intermediate') (FS), and homozygotic slow (SS) where F (fast) and S (Slow) are generic for the different fast and slow alleles.

Several diseases have been associated with the acetylator pheno- and/or genotype. The best documented are bladder carcinoma (slow),[8,9] colorectal adenomata (fast),[10,11] Gilbert's Syndrome (slow)[12], Type I Diabetes Mellitus (fast) and Type II Diabetes Mellitus (slow)[13] and familial Parkinson's Disease (slow)[14].

The question *therefore arises* whether the speed at which acetylation takes place in an individual plays a role in human lenticular opacification or whether acetylator geno- and phenotype may be genetic markers for age related cataract?

C THE HYPOTHESES THEREFORE ARE:

1. Lens opacities in a group of dyslipidemic patients develop at an earlier age than in the normal population and one or more of the components of the lipid profile is associated with this phenomenon.
2. Subjects manifesting the slow (S) acetylation geno- and phenotype are more prone to the development of cataracts than subjects expressing the fast (F) acetylation geno- and phenotype.

D SUMMARY OF MATERIALS AND TECHNIQUES

1. (Project 93/101) One hundred and fifteen (n = 115) adult patients of both genders, irrespective of race, with proven dyslipidemia, were prospectively studied. Appropriate clinical, ophthalmological and biochemical examinations and assessments were performed on all patients. Correlations were sought between the classic symptoms and signs of dyslipidemia in the trial population, and the incidence of lens opacification. The incidence in the trial population was statistically compared to the incidence in the population at large, in order to validate correlations that were observed.

2. (Project 97/067) One hundred and thirty nine (n = 139) adult patients of both genders, and of mixed race (Cape Coloured), presenting with age-related lens opacification of severity requiring surgical intervention to restore sight, were enrolled in the trial. Patients were excluded from the trial in the event that the acetylator genotype was non-concordant with the phenotype. The prevalence of lens opacities in each of the three different acetylator subgroups constituting the trial population, was statistically compared to the prevalence in the corresponding subgroups constituting the population at large. The observed differences in the prevalences between the corresponding subgroups were analysed statistically in order to establish the significance of the differences, and to validate observed correlations between acetylator status and the risk of cataractogenesis.

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CHAPTER 1

LENS ANATOMY AND PHYSIOLOGY RELATED TO CATARACTOGENESIS

A. INTRODUCTION

In order to enable the reader to appreciate this dissertation optimally the first chapter will present a brief overview of current widely accepted basic science concepts related to the vertebrate lens. The anatomical structure of the lens is described and physiological, metabolic and biochemical aspects relevant to this dissertation are briefly considered. Emphasis is placed on the physiological aspects of lens aging.
The lens is in several ways a unique organ and therefore at the end of this chapter all the unique properties of the vertebrate eye lens, supporting its highly specialized nature are listed and summarized.

B. ANATOMIC ASPECTS OF THE LENS

The lens consists of:

- The lens capsule;
- the lens epithelium and
- the lens cells or fibres.

THE LENS CAPSULE

The capsule completely envelops the lens and is unique in that its cells of origin are completely contained by it. The capsule is the basement membrane of the lens epithelium and is the thickest basement membrane in the body. It is much thicker anteriorly than posteriorly and both portions are thicker towards the periphery (equator) than at the poles (Fig 1). Because the epithelium is the secretory source of the basement membrane and situated anteriorly, the capsule thickens anteriorly with age[1]. By old age lens capsule thickness is about 14µm at the anterior pole and 21µm above and below the equator where the zonules are inserted[2]. Like other basal laminae, the capsule is rich in type IV collagen but also contains types I and III collagen.

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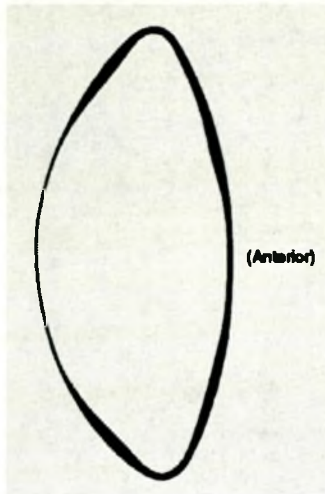

Figure 1: The lens Capsule

The capsule is freely permeable to water, ions and other small molecules, but offers a barrier to protein molecules the size of albumin and hemoglobin. No differences in permeability of capsules of normal and cataractous lenses have been noted[3].

THE LENS EPITHELIUM

The epithelium consists of a single sheet of cuboidal cells spread over the front of the lens, inside the capsule and extending outwards to the equator. Its cells are cuboidal in sagittal section, but polygonal in surface view. There are about 500 000 cells in the mature lens[4] with an increased density towards the periphery. This cell density declines with age.

The lens epithelium is divided into three zones:

- Central zone
- Intermediate zone
- Germinative zone

The *central zone* consists of a stable population of cells whose numbers gradually reduce with age. These cells do not normally mitose, but they can do so in response to damage.

The *intermediate zone* is peripheral to the central zone and its cells are smaller, more cylindrical and with a central nucleus. Mitoses are occasionally seen.

The *germinative zone* is the most peripheral and is located pre-equatorially only. It is the major site of cell division. From this region new cells migrate posteriorly to become lens fibres. Cytoskeletal proteins include actin, vimentin, microtubular protein, spectrin, alpha-actinin, and myosin. α-Crystallin is present, but not β and γ crystallin. The germinative zone is protected from the potentially harmful effects of radiant energy in the U-V range (300-400 nm) by its location behind the iris.

Gap junctions are found within the lateral membranes of contiguous cells, which permit the free movement of small molecules between them[5]. *Tight junctions* are virtually absent[6]. The resistance presented across the

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epithelium is therefore not high, and there does not appear to be a significant barrier to extracellular flow between the lens cells.

THE LENS FIBRES

Epithelial cells in the germinative zone elongate, the basal portion extends backwards along the inner surface of the capsule and forwards under the epithelium. Deposition of successive generations of lens fibres is associated with the formation of the nuclear bow (Fig 2).

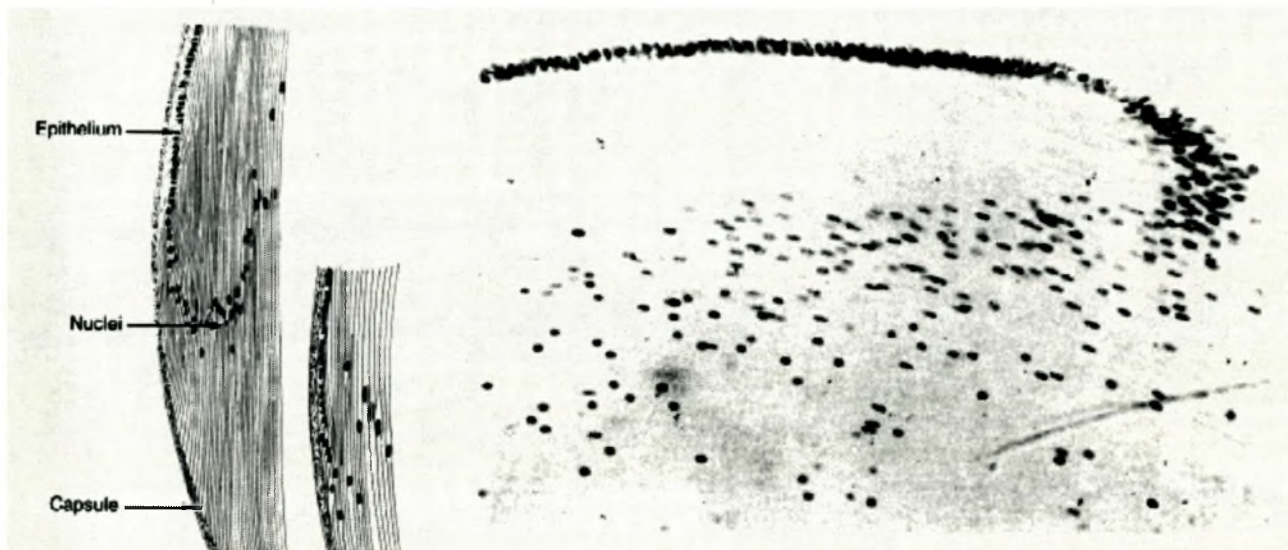

Figure 2: Lens fibre nuclear bow

The fibres are laid down in concentric layers, the outermost of which lie in the **cortex** of the lens and the innermost in the core or **nucleus.** The division between the cortex and the nucleus of the lens is arbitrary and for convenience may be taken to be the junction between fetal and postnatal lens fibres.

The fibres are strap-like or spindle-shaped cells which arch over the lens in concentric layers from front to back. They are hexagonal in equatorial cross-section and in the cortex can be seen to form radial rows (Fig 3).

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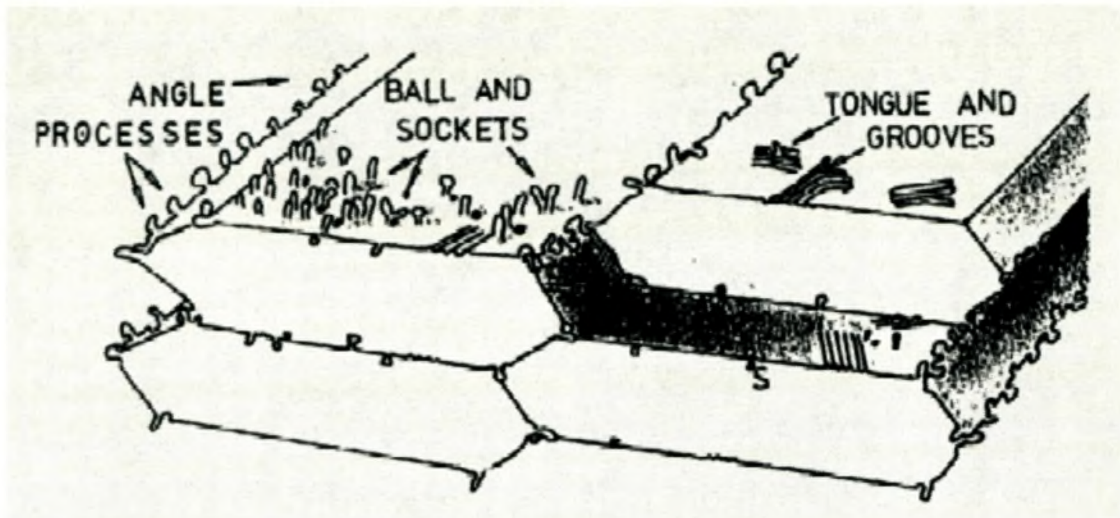

Figure 3: Form and junctions of cortical fibres

The youngest lens fibres, in contrast to the parent epithelium, show a denser amorphous granular cytoplasm. This cytoplasmic appearance is due to a high concentration of protein in the lens fibres, the fibres containing both beta and gamma crystallins in addition to the alpha crystallin present in the lens epithelium. Lens fibres contain the highest protein content of any cell in the body, about 35% of its wet weight being contributed by protein[7].

GAP JUNCTIONS

The extensive system of low-resistance gap junctions between the lens fiber cells is a unique feature of the lens. The dense packing of lens fibers connected by low-resistance gap junctions allows the tissue to function like a syncytium rather than a collection of individual cells. This arrangement has considerable impact on the metabolism and physiology of the lens.

Gap junctions are found in lens fibres. They are thought to fulfill two roles. *First* by conjoining large areas of membrane they further contribute to fibre order and therefore transparency[8]. *Secondly*, they are considered to contribute to lens function by providing pathways between terminally differentiated lens fibres, which lack the cellular organelles for metabolic co-operation.

Gap junctions are of two types:

- Crystalline (or high resistance) – found in association with lens epithelial cells.
- Non-crystalline (or low-resistance) – found between lens fibres.

The result of this is that the interiors of all the lens fibres exist in a state of relatively open communication with each other, and that the levels of concentration of ions and small molecules in individual fibres rapidly equilibrate.

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C. ASPECTS OF LENS PHYSIOLOGY AND METABOLISM PERTAINING TO CATARACTOGENESIS AND AGING

The lens is in several ways a unique organ. It is completely devoid of blood supply, has no innervation, grows in size and weight throughout life, sheds no cells and has a metabolism that is directed entirely toward the maintenance of its transparency.

COMPOSITION OF THE LENS

Compared to most other tissues, the lens has a particularly high protein and low water content. The high protein concentration is necessary for the lens to maintain a high refractive index. Protein accounts for more than one third of the lens wet mass, the other two thirds being water. The other constituents of the lens, including lipids, amino acids, a variety of peptides, carbohydrates and electrolytes make up only 1% of the lens wet mass.

TRANSPARENCY

In order to focus an image on the retina, the lens, in addition to being highly refractive, must be transparent. The transparency of the lens is largely the result of the highly ordered arrangement of the macromolecular components of the lens cells and the small differences in refractive index between light-scattering components.
The normal lens is not perfectly transparent but scatters about 5% of the light falling upon it. More than half of this light scattering is due to lens cell membranes, even though the cell membranes represent a volume fraction of only 0.05.
Transparency of the lens is highly dependent on protein order and structural integrity. Relatively small changes in any of these parameters may lead to the development of lenticular opacification and later cataract. Such changes in the lens include aggregation, changes in tissue hydration, phase separation of molecular components, breakdown of cell membranes and changes in the structure of the cytoskeleton.

LENS METABOLISM

The metabolism of the lens is entirely directed toward the maintenance of transparency. Regulation of lens electrolyte balance serves to maintain the normal hydration of the lens, which is a critical feature in lens transparency. Protection of the lens from oxidative damage is also critical and there is a sophisticated set of biochemical pathways to preserve the oxidative status of the lens.
The main location of lens metabolism is located in the lens epithelium. The elaborate system of gap junctions allows cells deep within the lens to communicate with the outer cell layers.

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Metabolism in the lens will be reviewed with reference to the following components:

C.1. Carbohydrate and energy metabolism;

C.2. Lens proteins;

C.3. Lens lipids;

C.4. Water and electrolyte balance;

C.5. Non-electrolyte transport mechanisms;

C.6. Oxidation and reduction pathways.

C.1. CARBOHYDRATE AND ENERGY METABOLISM

Energy production in the lens is almost entirely dependent upon the metabolism of glucose. Glucose and a number of sugars enter the lens by simple diffusion assisted by a mediated transfer process[9] . The level of free glucose in the lens is less than one tenth of that in aqueous humor suggesting rapid metabolism once glucose enters the lens. The lens derives more than 70% of its energy through **anaerobic** glycolysis. The **aerobic** metabolism of glucose via the Krebs cycle is limited to the lens epithelium.

Anaerobic glycolysis renders only two moles of ATP for every mole of glucose. It is therefore not as efficient as the aerobic metabolism of glucose, but its preponderance in the lens avoids the problem of oxygen starvation, since the lens is devoid of a blood supply and is dependent on the rather low oxygen tension present in the aqueous humor. Lenses can survive in organ culture in completely anaerobic conditions as long as there is an adequate supply of glucose. When excess glucose is present, it enters the sorbitol pathway with its damaging consequences.

Aerobic metabolism of glucose is much more efficient than glycolysis, since it produces 38 moles of ATP from each mole of glucose. The production of ATP in the lens via the Krebs cycle is limited to the epithelium and only 3% of lens glucose is metabolized this way. Because of the efficiency of the Krebs cycle, this generates up to 20% of the total ATP needed in the lens[10].

Hexosemonophosphate shunt. In addition to glycolysis and the Krebs cycle the lens also metabolizes glucose via the hexosemonophosphate shunt[11]. This shunt does not generate a large quantity of ATP, but is an important source of NADPH, which is critical to the sorbitol pathway and the enzyme glutathione reductase. Thus the hexosemonophosphate shunt is linked to sugar cataract.

The **Sorbitol pathway** of the lens converts glucose to sorbitol using the enzyme aldose reductase and then to fructose using polyol dehydrogenase. Under normal conditions the sorbitol pathway accounts for only some 5% of the metabolism of glucose by the lens. Aldose reductase is the first enzyme in the sorbitol pathway and is readily activated when there is only a moderate increase in glucose levels in the lens. Aldose reductase together with NADPH converts glucose to sorbitol, which accumulates within the cells of the lens. Since the cell membranes are relatively impermeable to sorbitol, it cannot diffuse out and an osmotic gradient develops that induces an influx of water and results in lens swelling.

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C.2. LENS PROTEINS

35% of the wet weight of the lens (nearly double that found in other tissues) is protein. Lens proteins can be separated into two classes based upon their solubility in water. The **water-soluble** lens *crystallins* account for nearly 90% of the total lens proteins. The **water-insoluble** 10% consist of *membrane proteins, cytoskeletal proteins*, and *aggregated crystallins.* Crystallins are not detected outside of the lens.

LENS CRYSTALLINS

The lens crystallins are a heterogeneous group of structural proteins identified as alpha, beta, and gamma crystallin.

Alpha crystallin. This is the largest of the crystallins (mol. weight 1 x 10^3 kDa). It accounts for only 35% of the total lens protein. Alpha crystallin is not a single protein. It is composed of four polypeptide subunits: alpha A1 and A2, and alpha B1 and B2. It has been shown that the subunits A2 and B2 are primary products of gene translation, while A1 and B1 are post-translational products of A2 and B2. The latter are found only in the epithelium, while A1 and B1 are found only in the lens fibers. As the lens ages, larger aggregates are formed with molecular weights as high as 50 x 10^3 kDa, contributing to light scattering and later cataract.

Beta crystallin. This is the most abundant water-soluble protein, representing about 55% of the total lens protein (mol. weight 40-250 kDa). They are therefore the most heterogeneous group of the crystallins because of the variation in size. Four distinct subgroups exist based upon the molecular weight.

Gamma crystallin. This is the smallest (mol. weight 20-27 kDa) and least abundant of the crystallins (only about 1-2% of the total). They precipitate when the lens temperature is lowered to 10°C resulting in what has been termed the cold cataract. Rewarming permits solubilization of the protein and transparency.[12]

WATER-INSOLUBLE PROTEIN

These are derived from membrane proteins. Proteins that are an integral part of the lens cell membrane are called *intrinsic* membrane proteins, while those associated only with the membrane surface are called *extrinsic* membrane proteins. The main *intrinsic* membrane protein is a 26 kDa polypeptide, which is thought to be a principal component of lens gap junctions. This polypeptide is degraded to a 22 kDa protein with aging. Gap junctions have been shown to contain high cholesterol and sphingomyelin content[13].

PROTEIN SYNTHESIS. PROTEOLYSIS. AND AGING

The **biosynthesis** of proteins in the lens, involves the transfer of genetic information contained within DNA via messenger RNA to the ribosomes of the cells that generate the polypeptide chains. This is the exact same process like in all other tissues. ATP derived from carbohydrate metabolism supplies the necessary energy. Protein synthesis takes place predominantly in the lens epithelium and the outer layers that contain

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the necessary intracellular organelles. McAvoy[14] suggests that two synthetic compartments, one an anterior proliferation compartment and the second an elongation compartment exists. Only α crystallin was detected in the epithelial cells of the first compartment but all three crystallins were found in the elongating compartment.

Aging. Lens proteins undergo substantial molecular modification with aging[15]. With aging soluble lens proteins (crystallins) *aggregate* thereby generating high-molecular-weight species that become water insoluble. By the age of 20, about 15% of the lens protein has become insoluble. By the 6th decade this has increased to 50%.[16] These aggregates can contain all three crystallins and can have molecular weights up to 50 million Daltons. In cataract the aggregate that appears results from disulfide bonds.

Carbohydrate molecules can also attach themselves directly to the protein amino acids of the lens, which favours protein aggregation. This process is known as *nonenzymatic glycation.*

Lens proteins also undergo *proteolysis*[17]. Both exopeptidases and endopeptidases (proteolytic enzymes) accumulate in the lens with aging. These proteases are probably responsible for the degradation of proteins that become damaged during aging.

C.3. LENS LIPIDS

Lens lipids include cholesterol, phospholipids, and glycosphingolipids. Most of them are associated with cell membranes and therefore found in a protein lipid complex. About 50 to 60% of the lens lipid is cholesterol. This concentration is so high that the ratio of cholesterol to phospholipid in the human lens is the greatest known[18]. The major phospholipid associated with the human lens cell membrane is sphingomyelin. The high cholesterol content coupled with sphingomyelin makes the lens cell membranes quite rigid. This rigidity appears to increase with aging[19]. Furthermore, the lens epithelium can metabolize arachidonic acid via the cyclooxygenase pathway to prostanoid compounds[20], but there is no evidence for a lipoxygenase pathway[21].

With **aging** substantial changes take place in lipid composition and distribution[22]. From about age 25 to 75 there is a doubling of lens cholesterol and a concomitant increase in sphingomyelin. On the other hand lens lipid metabolites, phosphatidylethanolamine and phosphatidylcholine decrease with aging. These changes, which reflect alteration in cell membrane structure, might be expected to have a considerable impact upon lens cell membrane function as the lens ages.

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C.4. WATER AND ELECTROLYTE BALANCE

Maintenance of lens hydration is critical to lens transparency.

WATER

The adult human lens is approximately 65% water. This represents relatively low water content. Because the cells of the lens are tightly packed, there is only a very small extracellular space. Regulation of intercellular water is determined largely by the distribution of monovalent cations (K^+ and Na^+).

MONOVALENT CATION BALANCE (SODIUM AND POTASSIUM)

The sodium and potassium of the whole lens is similar to that of a single cell. It is now well appreciated that sodium, potassium, chloride, and many other small molecules freely enter and leave the lens.[23] This process is governed principally by an active transport system located in the lens epithelium.[24]

DIVALENT CATION (CALCIUM AND MAGNESIUM) HOMEOSTASIS

Calcium is present in the lens at levels more than 50 times lower than that found in aqueous humor, suggesting that there must be a special transport mechanism to exclude calcium from the lens. There is strong evidence for a *calcium ATPase* in the lens[25,26]. Increased concentrations of calcium are cytotoxic in the lens and thought to contribute to the development of cataract, but it is not yet known whether the calcium transport system is impaired during cataract development. Studies have however suggested that calcium ATPase is very sensitive to oxidative damage, in vitro[27].

Magnesium functions as a cofactor in a number of enzyme reactions, but the level of magnesium in the lens changes little in human or experimental cataract.

C.5. NON-ELECTROLYTE TRANSPORT MECHANISMS

Only the transport of amino acids and ascorbic acid will be considered.

AMINO ACIDS

A continual supply of amino acids is essential to the lens in order to maintain uninterrupted protein synthesis. Amino acids are actively transported into the lens such that their concentration in the lens generally exceeds that in the aqueous humor. The site of amino acid transport is the lens epithelium. Different transport mechanisms exist for the different amino acids e.g. one each for alanine, leucine, glycine, and taurine[28]. Amino acid transport in the lens appears to be dependent upon the sodium gradient generated by Na,K-

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ATPase. The high sodium content found in human cataracts markedly interferes with amino acid uptake. Reduced amino acid transport accompanies aging and several forms of experimental cataract[58].

ASCORBIC ACID

Studies have suggested that the lens possesses a carrier-mediated transport system to accumulate ascorbic acid[29,30,31]. Of particular interest is the role of ascorbic acid as a scavenger of free radicals. However, ascorbic acid can also be pro-oxidant, since together with light and the presence of a metal ion, it will generate hydrogen peroxide.

C.6. OXIDATION AND REDUCTION PATHWAYS

Oxidation-reduction mechanisms have special importance in the lens. Oxidative damage can result in a number of molecular changes that contribute to the development of cataract. The lens must therefore possess efficient reducing systems, as well as detoxification enzymes such as catalase and superoxide dismutase[32].

Gluthathione plays a central role in protecting the lens from oxidative insult and in the process glutathione is converted into its oxidized form. Gluthathione is a tripeptide that is synthesized in the lens[33]. The enzymes responsible for glutathione synthesis have been shown to decrease in human senile cataract[68]. The concentration of glutathione also falls in virtually all forms of cataract, both human and experimental, thus leaving the lens even more vulnerable to oxidative insult.

The following processess also protect the lens from oxidative insult:

There are small amounts of ***catalase*** in the lens that converts hydrogen peroxide to water and oxygen[34].

Hydrogen peroxide is also detoxified by ***glutathione peroxidase***, a reaction in which glutathione serves as a cofactor.

Superoxide radicals are detoxified by the enzyme ***superoxide dismutase*** in the lens.[35]

Protein disulfide bonds can be reduced by the ***thio-redoxin system*** and can therefore contribute to the protection of thiol groups in the lens.

A family of enzymes called ***thiol transferases***, which involve glutathione as a cofactor can also protect the thiol groups in the lens.

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D. WHAT IS UNIQUE ABOUT THE LENS?

The vertebrate eye lens is a highly specialized organ whose sole function is to carry out proper refraction of incident light beams in order to ensure visual acuity. The organ, which is completely devoid of blood vessels, gets its nourishment from the surrounding fluid, the aqueous humor. This organ has several properties, which makes it one of the most unique organs in the human body.

1. Unlike all other organs, the lens *continues growing* throughout the lifespan of the organism.[36]
2. The lens has *no nerves or blood supply.* Nutrition and removal of breakdown products take place through the aqueous humour. It also has a limited capacity for repair.
3. The lens is the only organ that *never sheds any cells.* This could aid in explaining the growthrate throughout life.[37]
4. The *cortex* of the adult human lens is *replaced by new growth* approximately *every 5 years.*[38]
5. The lens is an elegantly simple tissue. It is made of *only two types of cells: epithelial cells,* which have not yet completely differentiated and not yet elaborated the major gene products, *and fiber cells*, in which these processes have been initiated or even completed.[39] The lens fibres comprise more than 90% of the lens bulk. Between these lens fiber cells is a unique and extensive system of low-resistance gap junctions.[40]
6. The lens *capsule* is an acellular and elastic structure and is analogous to basement membrane. It is the *thickest basement membrane* in the body.[41] The principal composition of the capsule is type IV collagen.
7. The lens is one of the *slowest metabolizing* tissues in the body.[42]
8. The outer epithelial monolayer contains the only cell population that has *mitotic activity.*[43]
9. The cell membrane of the human lens contains the *highest relative concentration of cholesterol* in nature.[44] The concentration of cholesterol in the lens is extraordinarily high, so that the ratio of cholesterol to phospholipid in the human lens is the greatest known.[45]
10. The concentration of *ascorbic acid* in most of the ocular tissues including the lens is substantially higher in comparison to other bodily tissues. The high intraocular concentration of ascorbate is maintained by an active transport of ascorbate from the plasma to the aqueous across the blood aqueous barrier, maintaining an approximately 20x higher concentration in the latter. (E.g. serum 10mg/L compared to 200mg/L in aqueous and 250mg/kg of the wet tissue weight of the lens.)[46]
11. The solid mass of the lens is uniquely about *98% protein.*[47] Two-thirds of the molecular makeup is water and one-third protein (35%), nearly double that found in other tissues. Other constituents represent only 1% of the total lens wet weight.[48] This high protein content is necessary for the lens to have a high refractive index, allowing it to bend light rays into focus onto the retina.
12. As far as aging of proteins is concerned, there is the general phenomenon of loss of material, which implies an imbalance between biosynthesis and breakdown. The human eye lens, however, forms an exception to that rule since the *intra-cellular protein level is virtually maintained* during the *whole life span* of the person. The lens provides an especially useful system for the study of aging.because proteins present in the nucleus of the adult lens have been synthesized during fetal life.[49]

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13. *α-Crystallin* is one of the major vertebrate lens proteins. Due to its long life in the eye lens, α-crystallin is one of the *best-studied proteins* with respect to post-translational modifications, including age-induced alterations. Many attempts have been made to crystallize α-crystallin, but no crystals have been obtained up till now.[50]

14. α-Crystallin is a rather unique eukaryotic protein in that its N-terminal methionine residue, donated by the initiator tRNA and which *becomes acetylated* during peptide growth, is not removed from the polypeptide chain. The only other hitherto known N-terminal acetylated methionine residue occurs in the coat protein of turnip yellow mosaic virus particles and in tropomysosin from rabbit muscle.[51]

15. The intrinsic *structural stability of α-crystallin* makes it suitable to reside in the lens life-long and without turnover. By preventing undesirable protein interactions and refolding unfolded proteins, it may contribute to the maintenance of lens transparency and integrity. Actually, the constitutively high level of α-crystallin in the lens might make this organ *permanently stress-tolerant.*[52]

16. Lens extraction is the *most frequently performed surgical procedure* in the world and the costs associated with lens problems comprise the largest line item in the Medicare budget in the United Kingdom[53]. In the USA more than two million lens extractions are performed annually with the attendant significant health care costs (US $ 5 billion).[54]

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CHAPTER 2

CATARACT ETIOLOGY – A REVIEW

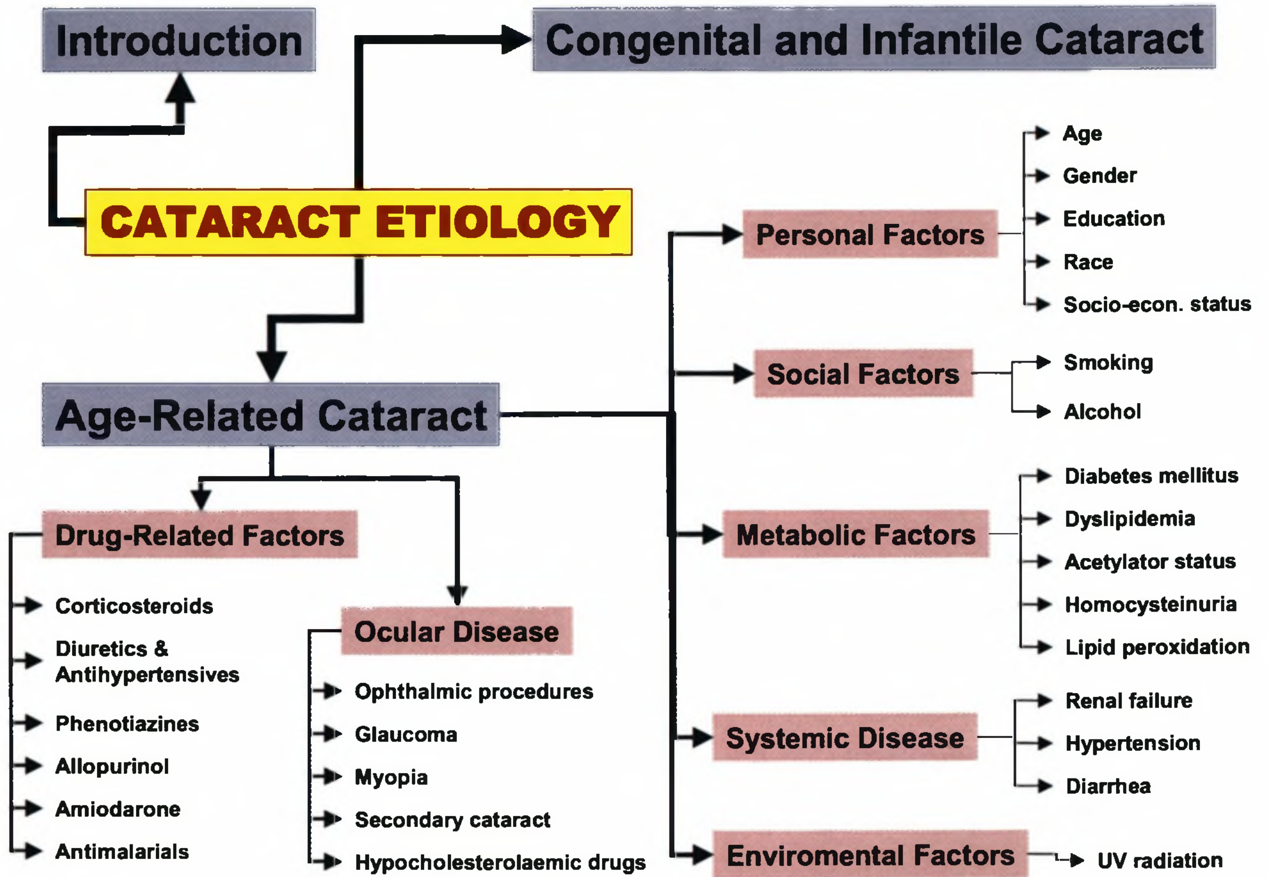

Figure 1: Classification of the etiological factors discussed in this chapter

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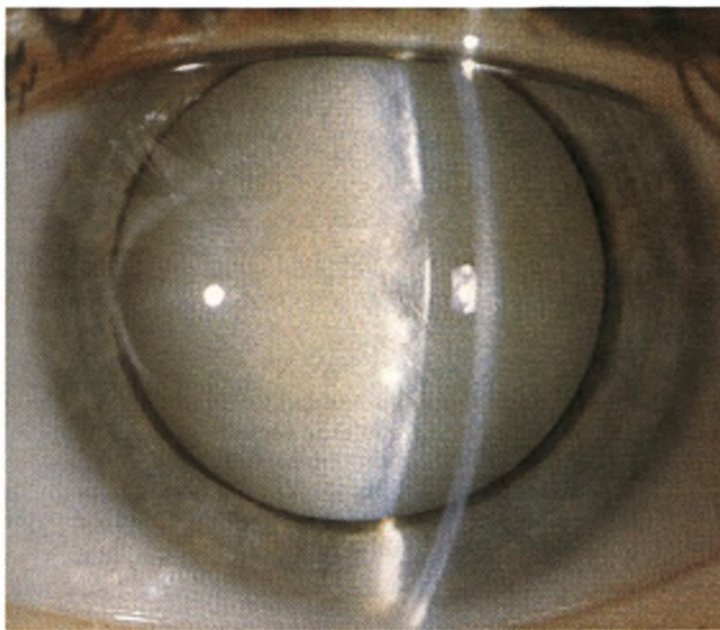

Figure 2[1]: Mature age related cataract

A. INTRODUCTION

Comprehensive recent reviews on the etiology of cataract are lacking from the scientific literature. The following review is an updated overview of the significant known etiological factors in human cataractogenesis. This review focuses on adult cataract and deliberately omits congenital and infantile cataract. Factors of a general nature are discussed e.g. personal, social and environmental, followed by disease (both systemic and ocular) and therapy associated factors. Mechanisms are elucidated wherever possible.

The noun **cataract** is derived from the Latin *cataracta* and from the Greek *katarraktes (probably from katarassein, to dash down:* kat-, kata-, cata- + arassein, *to strike.)*, which denotes a large or high waterfall, a great downpour, a deluge or a portcullis. Analogously a cataract is a complete or partial opacification of sufficient severity, on or in the human lens or capsule, to impair vision.[2]

Proper vision is achieved by a series of eye tissues working harmoniously in concert. Most eye debilities involve dysfunction in the lens or retina, and hence this chapter will focus on and elucidate etiological factors that may affect the proper functioning of the lens as target organ.

It is now well established that cataract formation is a multifactorial disease. Several of the etiological factors are constitutional and hence difficult to manipulate. Others are environmental in nature and a little easier to control whilst a significant number are behavioural in nature and fall well within the individual's own ability to control or modify.

Although this review focuses on risk factors involved in lens opacification in adults, for perspective a brief mention is made of the etiology congenital and infantile cataracts.

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Congenital cataract is numerically the most important cause of remediable blindness in children, being far more common than, for example, retinoblastoma or congenital glaucoma.[3]

The prevalence of infantile cataracts has been reported to be in the order of between 1.2 and 6 cases per 10,000 births.[4,5,6,7] Furthermore it has been estimated that between 10% and 38.8% of all blindness in children is caused by congenital cataracts and that one out of every 250 newborns (0.4%) has some form of congenital cataract.[8]

This review will not elaborate on this topic, but will focus on adult cataract etiology.

B. ADULT CATARACT

B.1. PERSONAL FACTORS

B.1.1. GENDER

It has often been observed that more females than males have cataracts and undergo cataract surgery. This is partly explicable by the longer life span of women and therefore their over-representation in the age groups which cataract is most common. It does appear however that there is an additional effect: a true excess risk of cataract in females.[9] In Nepal the prevalence of cataract was greater in females than in males throughout the age range[10] and the overall risk ratio was found to be male:female, 1:1.4. In most case-control studies the two groups were age- and sex-matched so that the effect of sex could not be explored. Hiller et al had to combine the results from three earlier studies in the United States and India to find a significant excess relative risk in females:-male:females, of 1:1.13. The follow-up study of data from the National Health and Nutrition Examination Survey (NHANES) further suggested that such an excess risk for women is specific to cortical cataracts.[11] In a population-based prevalence survey in Beaver Dam, Wisconsin, women had more cortical opacities compared to men within similar age groups.[12] The Beaver Dam Study reported a protective effect for nuclear opacities with current use of postmenopausal estrogens[13]. The Epidemiology of Cataract in Australia Study found that a protective relationship of hormone replacement therapy and cortical cataract exists at the univariate level, but that this relationship was not significant in multivariate analyses.[14] Nuclear cataract cases were more likely to be female in the above study, even after age adjustment. The study was however unable to support the hypothesis that hormone replacement therapy is protective against nuclear cataract.

B.1.2. BODY MASS INDEX

Body Mass Index (BMI) is computed as weight in kilograms divided by the square of the height in meters (kg/m^2) and is frequently identified as a risk factor for cataract, but the nature of the association is

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unclear. Several mechanisms may play a role:

1. BMI affects *glucose* levels, which are associated with increased risk of cataract;
2. Higher BMI also increases *uric acid* concentrations and the risk of gout, which were associated with cataract in some studies;
3. BMI is an important determinant of *hypertension* which, in turn, is thought by some to be associated with cataract.[15]

Low BMI[16, 17] have been thought to be associated with cataracts in some developing countries. Hankinson et al[18] in a prospective study examined the association of BMI with cataract extraction in a large cohort of women and found elevated rates of cataract in those with higher BMI. Women with BMI of 23 or above had significantly elevated rates of extraction, between 46% and 65% higher than those with body mass index less than 21. Glynn et al in a prospective cohort study of a total of 17764 apparently healthy US male physicians aged 40 to 84 years who were free of cataract at baseline were followed for 5 years.[19] In this group higher body mass index was especially strongly related to risk of posterior subcapsular and nuclear sclerotic cataracts and was also significantly related to risk of cataract extraction. Furthermore BMI below 22 appeared especially protective against posterior subcapsular cataract, with reductions in risk of 50% or more relative to each of the groups with a higher BMI. They concluded that BMI appears to be a strong and independent risk factor for cataract in this well-nourished and socioeconomically homogeneous study population.

Cataract due to suboptimal BMI is potentially preventable as body mass is modifiable, cataract caused by overweight is therefore potentially preventable.

B.1.3. SOCIO-ECONOMIC STATUS

In population studies less education and lower income have consistently been associated with impaired vision and cataract[20, 21]. The relationship of education, income, marital status, and employment status to age-related cataract, and impaired vision was addressed in the population-based Beaver Dam Eye Study.[22] While controlling for age and sex in this study, less education was significantly ($p< 0.05$) related to higher frequency of nuclear and cortical cataracts. Lower reported total household income was significantly associated with higher frequencies of cortical and posterior subcapsular cataract. This relationship between total household income and cataracts was observed in both men and women.

Less education has been associated with higher frequencies of a history of heavy drinking, cigarette smoking, and less vitamin supplement intake, all of which have been found to be related to specific types of cataract.[23, 24]

Marital status is a measure of social support, which is postulated to be an important factor in developing, and managing complications associated with disease.[25] While controlling for age and sex, people who were never married had a higher frequency of impaired vision than those currently married. This may be due to the fact that married people may have more social pressure to seek health care and to maintain

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familial responsibilities, and they may have more transportation assistance than their unatached counterparts.

In summary, less education and income are positively related to cataract and visual impairment.

B.2. SOCIAL FACTORS

B.2.1. SMOKING

Tobacco is the leading preventable cause of disease, disability and premature death.[26]

Of the 4 000 active substances in tobacco smoke, most are hazardous to human health[27]. More than 40 of these chemicals are carcinogens and many others are deleterious to the cardiovascular and the pulmonary systems. They include nicotine, tars, nitrosamines, polycyclic aromatic hydrocarbons, hydrogen cyanide, formaldehyde, and carbon monoxide.[28] Cigarette smoking is also a substantial source of intake of heavy metals and toxic mineral elements, such as cadmium, aluminum, lead, and mercury, all known to be poisonous in high concentrations.[29]

Tobacco smoke furthermore contains numerous compounds with oxidative properties, the existence of which is linked to the pathogenesis of several of the most common eye disorders, such as cataract and age-related macular degeneration.

Table 1 summarizes 4 very thought-provoking studies all supporting the view that smoking is associated with the development of cataract.

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Table 1: Smoking and the risk of cataract

Study:	Relative risk (RR)	95% CI	Comments
Leske et al, 1991[30]	1.68	0.96-1.94	Association with nuclear cataract
Hankinson et al, 1992[31]	1.63	1.8-2.26	Conducted on 50,828 women; RR for developing posterior subcapsular cataract is 2.59
Christen et al, 1992[32]	2.16	1.46-3.20	N = 22,071 males; RR for nuclear cataract is 2.24 and for posterior subcapsular, 3.17
West et al,1995[33]	2.40	1.00-6.00	Conducted on 442 watermen of the Chesapeake Bay

Nuclear sclerosis appears to be the type of cataract most commonly associated with smoking.

- **MECHANISM**

Cigarette smoking is thought to cause cataract through its effect on the oxidant-antioxidant status of the lens. Oxidative damage plays a major role in cataractogenesis.[34,35] Animal, laboratory, clinical, and epidemiological data support the relationship between cataract prevention and diets rich in nutritional factors with antioxidant properties, such as riboflavin, vitamins C and E, and the carotenoids.[36]
Smoking appears to further impair lens function by imposing an additional oxidative challenge as well as by contributing to the depletion of endogenous anti-oxidant pools.[37, 38] Tobacco smoke also contains large amounts of heavy metals, such as cadmium, lead and copper, which appear to accumulate in the lens and exert further toxicity.[39]

The above data strongly support an association between tobacco smoking and cataract formation. Given the magnitude and seriousness of the cataract problem, an important preventive measure in fighting this disorder is to quit smoking. It is important to note that smoking is on the increase in the developing world, where cataract surgery is not always readily available.

B.2.2. <u>ALCOHOL</u>

Some studies[40,41] have reported a relationship between alcohol consumption and cataract, while other studies[42,43] have found no relationship. One study[44] reported that both abstainers and heavy drinkers were more likely to have cataract than moderate users, while another[45] found that total abstainers were more likely to have cataract than alcohol users.

As far back as 1973 Sabiston[46] studied 40 patients in New Zealand over a 5-year period. They found a definite correlation between high alcohol intake, Dupuytren's contracture, and the development of cataracts. Although they could not explain the mechanism of cataractogenesis, they concluded that an element of alcohol induced chronic dehydration was an important factor. In New Zealand, heavy drinking often commenced with the ingestion of large quantities of beer. The national average

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consumption of beer there is 100 liters per head annually, with manual laborers ingesting a daily total of 4 liters of beer per person per day on average. These persons were almost invariably heavy cigarette smokers as well. He further noted that the cataracts commenced in a posterior subcapsular position, and could progress to almost full maturity in six months. There was almost universally a history of heavy cigarette smoking as well. Malnutrition was only sometimes seen.

Drews[47] in 1970 also drew attention to the association of ethanol and cataract. Two decades later he writes: "A patient in his or her 40s or 50s who appears with a posterior subcapsular cataract should be investigated for alcoholism...In my practice, about 25% of patients younger than age 65 years who present with cataract are found to be alcoholic on careful investigation. It has been my experience that if the opacities are incipient and if the consumption of alcohol is stopped completely, the posterior subcapsular changes may reverse and even disappear.[48]

Alcohol has many metabolic effects, and modifies the absorption of drugs and dietary components. These effects may be important in the alcohol-cataract relationship. However, one cannot exclude the possibility that alcohol itself, especially when consumed in high volume, may be a direct toxin to the human lens.[49]

B.3. METABOLIC FACTORS

B.3.1. DIABETES MELLITUS

Juvenile Diabetic Cataract, classically known as the "snowflake cataract" is now uncommon with the advent of effective hypoglycemic therapy. It occurs in insulin dependant diabetics in whom onset was before the age of 30[50]. Other typical features of this type of cataract are subcapsular and cortical 'snow flakes', and polychromatic opacities and vacuoles. These may proceed to mature cataracts within weeks or months and, rarely, may be reversible after normalization of blood glucose over some weeks or even as rapidly as 24 hours[51, 52].

- **CATARACT IN DIABETIC ADULTS**

Cataract has a greater prevalence in diabetics with a greater risk of development in women, and is dependent on the duration of diabetes. The morphology is no different from that of age-related cataract, although the frequency of some subtypes is increased. The major features are nuclear cataract, cortical spokes and posterior subcapsular cataract. Cataract is the second most common cause of severe visual loss in adult onset diabetics[53]. Various other reports have shown an association between cataract, and diabetes duration[54]. The Framingham study showed a significant excess risk in the 50 – 64 year age group (relative risk 4.02), while the HANES study showed a relative risk of 2.97 in this age group. A case-control study in Oxford found an increased risk for cataract extraction in diabetics in the age group 50 – 79 years.[55]

In the Lens Opacity Case Control study[56], diabetes increased the risk of posterior subcapsular, cortical and mixed forms of cataract.

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It has been suggested that the increased nuclear scattering and brunescence in diabetic lenses is likely to be the result of increased glycation and the formation of advanced glycation end products.[57].

Cortical cataract can experimentally be caused by agents which interfere with membrane permeability, ion and water control. Hydrogen peroxide is present in normal human aqueous humor, and is present in raised levels in the aqueous of patients with cataract[58]. Higher levels are found in the aqueous of diabetic patients with cataract. Simonelli et al[59] have also shown an increase in malondialdehyde in cataractous compared with non-cataractous lenses, which is greater in the cataracts of diabetic patients. Malondialdehyde is a product of lipid peroxidation of cell membranes, and is regarded as an indicator of oxidative membrane damage. It has been noted that oxidative stress may cause lens membrane damage experimentally[60]. It may also cause damage to DNA. Subcapsular cataract may reflect aberrations of lens mitosis and lens fibre differentiation, and in turn, could be the result of oxidative damage.

B.3.2. DYSLIPIDEMIA

The relationship between cholesterol and cardiovascular disease is well documented. The relationship between cholesterol and lens opacity is, however, far less well appreciated.

Issues relating to drug safety and inherited defects in enzymes mediating cholesterol metabolism have brought renewed attention to a possible inter-relationship between lipid metabolism and cataract induction in humans. It has been shown that inhibition of cholesterol synthesis in the lens leads to cataract formation in man[61].

Jahn et al [62] attempted to determine the role of glucose and lipid metabolism in the formation of cataract in elderly people undergoing cataract extraction. They found that patients with posterior subcapsular cataract had higher concentrations of fasting serum triglycerides and were significantly younger than patients with nuclear or cortical cataract. Their results furthermore suggest that the association of hypertriglyceridemia, hyperglycemia and obesity favours the formation of a specific morphologic type of lens opacity, posterior subcapsular cataract, occurring at an early age.

Issues regarding dyslipidemia and cataractogenesis are discussed and examined more comprehensively in chapters 3-5.

B.3.3. ACETYLATOR STATUS

The human acetylation polymorphism has thus far not yet been implicated in the pathogenesis of age-related cataract. The association of the conjugative acetylation status with a variety of disease states, nuclear lens opacification inter alia, would seem to indicate a possible etiological role for nitrogenous toxins in the pathogenesis of cataractogenesis. The possible role of xenobiotics, toxins, pesticides, preservatives, industrial chemicals and the human susceptibility to these substances (due to acetylator status polymorphism), is fully addressed in chapters 6 and 7.

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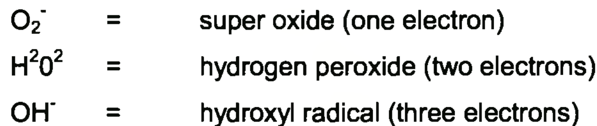
B.4. LIPID PEROXIDATION, FREE RADICALS, AND NUTRITIONAL INFLUENCES ON CATARACT FORMATION

Oxygen and oxygen-derived free radicals and a failure of intracellular calcium homeostatic mechanisms are recurring themes in a wide variety of cell injuries[63]. The addition of electrons to molecular oxygen leads to the formation of toxic free oxygen radicals or reactive oxygen species (ROS) e.g.



O_2^- = super oxide (one electron)

H^20^2 = hydrogen peroxide (two electrons)

OH^- = hydroxyl radical (three electrons)

These free radical species cause lipid peroxidation and other deleterious effects on cell structure. Recent studies have shown that lipid peroxidation, an event caused by imbalance between free radical production and antioxidant defense, may play a role in the genesis of cataracts[64,65]. Higher levels of malondiadehyde (MDA), a final product of the lipid peroxidation process, have been observed in diabetic and myopic cataracts[66] as apposed to senile cataracts. Protection of the cell against damage by these free radicals (ROS) takes place indirectly (enzymatically) by anti-oxidant enzymes: super oxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Direct protection is offered by mainly dietary anti-oxidants: ascorbate (Vit C), tocopherol (Vit E), carotenoids (Vit A) and glutathione (GSH)[67].

B.4.1. LIGHT AND OXYGEN AS RISK FACTORS FOR CATARACT

A number of epidemiological studies have confirmed an association between exposure to direct or indirect reflected UV light, and the risk for cataract formation.

Table 2: Extent of light exposure and the risk of cataract

Study:	Exposure		PR	95% CI
USA: NHANES survey[68]	Daily hours of sunlight in area; ages 65 -74	< 6.6h	1.0	
		7.1 – 7.7h	1.7	1.2 – 2.7
		>8.2h	2.7	1.6 – 4.6
Australia[69]	Daily hours of sunlight in area	<8h	1.0	
		8.5 – 9h	2.9	0.6 – 13.2
		>9.5h	4.2	0.9 – 18.9
Nepal[70]	Average hours of sunlight	7 – 9h	1.0	
		10 – 11h	1.2	0.9 – 1.4
		>12h	2.5	2.1 – 3.0

PR = prevalence ratio

CI = confidence interval

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Elevated levels of oxygen exposure perhaps show the clearest causal relationship between oxidative stress and cataract. Nuclear cataract was observed in patients treated with hyperbaric oxygen therapy[71], and markedly elevated levels of mature cataract were observed in mice that survived exposure to 100% oxygen twice weekly for 3 hours[72].

B.4.2. PROTECTIVE ROLE OF CELLULAR ANTIOXIDANTS AGAINST LENS DAMAGE

Protection of the organism against photo-oxidative insult can be viewed as two inter-related processes. Primary defenses offer protection of proteins and other lens constituents by lens antioxidants and antioxidant enzymes whereas secondary defenses include proteolytic and repair processes. The primary defenses shall form the focus our attention.

The major aqueous antioxidants in the lens are ascorbate[73] and reduced glutathione(GSH). [74]

B.4.2.1. ASCORBATE

Ascorbate is probably the most effective, least toxic antioxidant identified in mammalian systems. The following has been observed:

- The lens and aqueous concentrate ascorbate >10 times the level found in human plasma[75].
- Ascorbate levels in normal lenses are higher than in cataractous lenses[76].
- Increasing lens ascorbate concentrations by twofold is associated with protection against cataract-like damage[77].

A number of clinical, interventional and epidemiological studies have shown that a deficiency of Vit C in the diet is associated with lens opacification. [78, 79, 80]

B.4.2.2. GLUTATHIONE (GSH)

Reduced glutathione(GSH) levels in the lens are several folds higher than the levels found in whole blood and in plasma. GSH levels also diminish in the older and cataractous lenses[81].

B.4.2.3. VITAMIN E

Vitamin E is a lipid-soluble antioxidant that has the potential to inhibit lipid peroxidation[82] and appears to have a stabilizing affect on the cell membranes of the lens [83]. Supplementation of Vit E was associated with a decreased risk of lens opacification, in two different studies. Robertson and coworkers[84] found among age- and sex-matched cases and controls that the prevalence of advanced cataract was 56% lower (RR: 0.44: CI: 0.24-0.77) in persons who consumed vitamin E supplements (>400 I.U./day) than in persons not consuming supplements. Jacques and Chylack (unpublished) observed a 67% (RR: 0.33; CI:0.12-0.96) reduction in prevalence of cataract for vitamin E supplement users after adjusting for age, sex, race and diabetes.[85]

B.4.2.4. CAROTENOIDS

The carotenoids are a family of lipid-soluble compounds, occurring naturally in a variety of green and yellow colored vegetables and fruits, which have valuable anti-oxidant properties. Beta-carotene is the best-known carotenoid because of its importance as a vitamin A precursor. However it is only

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one of the 400 naturally occurring carotenoids[86] and other carotenoids may have similar or greater antioxidant potential. In addition to β-carotene, α-carotene, lutein and lycopene are important carotenoid components of the human diet[87].

Jacques and Chylack[88] were the first to observe that persons with carotene intakes above 18,700 IU/day had the same prevalence of cataract as those with intakes below 5,677 IU/day (RR:0.91; CI:0.23-3.78). Hankinson et al.[89] followed this report with a study that reported that the multivariate-adjusted rate of cataract surgery was about 30% lower (RR: 0.73; CI 0.55-0.97) for women with high carotene intakes (median = 14,558 IU/day) compared with women with low intakes of this nutrient (median = 2,935 IU/day). However, while cataract surgery was inversely associated with total carotene intake, it was not strongly associated with consumption of carotene-rich foods, such as carrots. Rather, cataract surgery was associated with lower intakes of foods such as spinach that are rich in lutein and xanthin carotenoids, rather than β-carotene. It is known that the human lens contains lutein and zeaxanthin but no β-carotene.

- **INTERVENTION STUDIES**

To date only one intervention trial designed to assess the effect of vitamin supplements on cataract risk has been completed[90]. Sperduto and co-workers[91] took advantage of two ongoing, randomized, double-blinded vitamin and cancer trials to assess the impact of vitamin supplements on cataract prevalence. The trials were conducted among almost 4,000 participants aged 45 to 74 years from rural communes in Linxian, China. Participants in one trial received either a multisupplement or placebo. In the second trial, a more complex factorial design was used to evaluate the effects of four different vitamin/mineral combinations. At the end of the five to six year follow-up, the investigators conducted eye examinations to determine the prevalence of cataract. In the first trial there was a significant 43% reduction in the prevalence of nuclear cataract for persons aged 65 to 74 years receiving the multi supplement (RR: 0.57; CI: 0.36-0.90). The second trial demonstrated a significantly reduced prevalence of nuclear cataract in persons receiving the riboflavin/niacin supplement relative to those persons not receiving the supplement (RR: 0.59; CI 0.45-0.79). However, the riboflavin/niacin supplement appeared to increase the risk of posterior subcapsular cataract (RR:2.64; CI: 1.31-5.35). The results further suggested a protective effect of the retinol/zinc supplement (RR: 0.77; CI: 0.58-1.02) and the vitamin C/molybdenum supplement (RR:0.78; CI: 0.50-1.04) on prevalence of nuclear cataract.

- **CONCLUSION**

Although light and oxygen are necessary for physiological function, when present in excess they seem to be causally related to cataracts. Aging might diminish the body's primary antioxidant reserves, antioxidant enzyme abilities, and diminished secondary defenses such as proteases.

The literature creates the strong impression that antioxidant intake might diminish the risk for cataract formation. Consequently, it is reasonable to assume that appropriate nutritional and educational intervention have the potential to promote our preventative goal. The preliminary data from the studies mentioned seemed to suggest that attention to correct nutrition may provide a practicable means of retarding the development, if not preventing, lens opacification and it's ultimate progression to cataract.

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B.5. OCULAR DISEASE

Many ocular diseases have been associated with cataract formation either as direct cause and effect relationships or as common associations.

B.5.1. MYOPIA

Weale[92]suggested that lenses of myopes are subject to excessive mechanical stress that could lead to cataract. Harding et al tested this hypothesis in a case-control study at Oxford and found that there was a twofold risk of cataract after the age of 50 in myopes.[93] Lim et al in the Blue Mountains Eye Study presented similar findings two decades later[94]. They concluded that early-onset myopia (before 20 years of age) may be a strong and independent risk factor for PSC cataract, that nuclear cataract was associated with presumed acquired myopia, whereas high myopia was associated with all three types of cataract. Conversely PSC cataract was inversely associated with hyperopia (OR 0.6; CI 0.4-0.9).

In the population based study of 3,271 Australians[95] an association between myopia of 1 diopter or more and both nuclear and cortical cataract was observed.

B.5.2. GLAUCOMA

Glaucoma has been shown to be strongly associated with the pathogenesis of cataract in many studies undertaken in many countries. The relative risk of cataract developing in a glaucoma patient can be as high as six times normal.[96] This risk more than doubles to an OR of 14.3 after glaucoma filtration surgery[97]. This rise in risk is most probably due to the trauma of surgery for glaucoma. Besides formal filtering procedures such as full thickness procedures, laser procedures for the management of different types of glaucomas are frequently performed. Each of these procedures carries the risk of inducing cataract, especially of the focal type.

Miotics, particularly long-acting cholinesterase inhibitors, if used long term, may cause tiny anterior subcapsular vacuoles and, occasionally, more advanced opacities. Cessation of medication may stop, retard or occasionally reverse their progression.[98]

B.5.3. OPHTHALMIC SURGICAL PROCEDURES

Many different ophthalmic procedures carry the risk of inducing cataracts. These include: surgical iridectomy, filtration surgery, corneal transplants, retinal detachment surgery as well as pars plana vitrectomy especially in diabetics. Assessing the surgical outcome in a series of 63 consecutive patients treated for rhegmatogenous retinal detachment by primary vitrectomy, Oshima[99] reported the

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reattachment rate by final examination as 100%, but there was a high incidence (53.8%) of cataract progression in phakic eyes.

More recently with the advent of minus power phakic IOL implantation surgery several reports have appeared of cataract induction secondary to the implantation of these lenses into the ciliary sulcus. Some have taken as short a time as 6 months, whilst others took 7 years to form.[100,101,102]

B.5.4. OCULAR TRAUMA

The development of cataract is a known complication following blunt or penetrating ocular trauma.

Crystalline lens subluxation, total dislocation, or localized cortical or diffuse opacities are often observed secondary to blunt ocular trauma. An unusual complication of blunt trauma is rupture of the posterior capsule with subsequent lens fibre hydration leading to rapidly progressive lens opacification. Posterior capsule breaks have been reported to develop thick, fibrous, opaque margins approximately 6 weeks after blunt trauma.[103]

B.5.5. SECONDARY CATARACT

B.5.5.1. UVEITIS

A secondary cataract develops as a result of some other primary ocular disease. The most common cause of secondary cataract is chronic anterior uveitis. The earliest finding is a polychromatic lustre at the posterior pole of the lens. If the uveitis is controlled, the progression of cataract may be arrested. If the inflammation cannot be controlled, anterior and posterior subcapsular opacities develop and the lens may become completely opaque.

B.5.5.2. HEREDITARY POSTERIOR SEGMENT DISEASE

Hereditary fundus dystrophies, such as retinitis pigmentosa, Leber's congenital amaurosis, gyrate atrophy, Wagner's and Stickler's syndromes may be associated with posterior subcapsular lens opacities[104]. In a study of 384 eyes in 192 patients with a mean age of 39.1 years who presented with typical retinitis pigmentosa, cataract was found in 46.4% of the eyes.[105]

Wagner's vitreoretinal degeneration is characteristically associated with high myopia, glaucoma, choroidal atrophy, retinal detachment and presenile cataract.[106]

Persistent hyperplastic primary vitreous (PHPV) is another congenital disorder that manifests as a range of ocular anomalies including leukocoria, microphthalmia, a retrolental fibrovascular membrane and cataract[107].

B.5.5.3. IRIS COLOR

McCarty et al, [108] in their Australian population study of 3,271 adults aged 40 years and older, found an association between cortical cataract and brown or dark brown irides for all ages that was not

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explained by country of birth or language spoken. In all age categories, brown iris color was also associated with nuclear cataract. No such association was found for posterior subcapsular cataract.

In the Italian-American Cataract Study, there was an increased, although not significant, risk of cortical cataract in people with brown irides[109]. In the National Health and Nutrition Examination Survey, blacks, which have dark brown irides, were found to have significantly increased risk of cortical cataract.[110] In both the above-mentioned studies dark iris color was also found to be a significant risk factor for nuclear cataract.

B.6. SYSTEMIC DISEASE

B.6.1. HYPERTENSION

The association between hypertension and cataract was first noted in the Framingham study in which earlier detection of elevated blood pressure was more common in those later found to have cataract[111]. Hypertension may be associated with high blood glucose, diabetes and other conditions known to predispose to cataract formation, not least of all the use of diuretics. Overall diuretic use was associated with an increased odds ratio(1.6) for cataract formation. Hypertension and diuretic consumption did not appear as risk factors in Oxford but the graded properties of different diuretics did emerge and with a similar sequence to that found in Edinburgh[112]. The only significant association of individual diuretics was an apparent protective effect by cyclopenthiazide and a risk associated with spironolactone which itself is a steroid. There was no significant association of particular sites of opacity with diuretic use[113].

B.6.2. DEHYDRATIONAL CRISIS

Harding has proposed that frequent episodes of diarrhea may be related to cataractogenesis and may account for the excess prevalence in some developing countries. Four intermediate events have been suggested to explain the role of diarrhea in the development of cataract:

1. Malnutrition secondary to malabsorption of nutrients;
2. relative alkalosis from administration of rehydrating fluids with bicarbonate;
3. dehydration induced osmotic disturbance between the lens and the aqueous humor and
4. increased levels of urea that may denature lens proteins by the process of carbamylation[114].

Six case-control studies have examined the relationship between severe diarrhea and increased risk of cataract, with discordant results. Two case-control, clinic-based studies, done in Hfadhya Pradesh[115] and Orissa[116], in India, have suggested a three- to four-fold increase in the risk for cataract for those with a history of episodes of life-threatening dehydration crises, severe enough to render the patient bedridden for at least three days. However, these findings were not replicated in two other epidemiologic investigations done in India. Using a less stringent definition of diarrhea (confinement to bed for one day), the India-US Case-Control Study found no associations with cataract.[117] An observational study

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done in Matiab, Bangladesh, revealed that diarrhea from all causes was not significantly associated with cataract, although it was difficult to determine how cataract was defined in the study.[118]

Considering the potential public health importance of diarrhea as a risk factor, as well as the biologically plausible role of dehydration in cataractogenesis, further research to clarify this association is needed.

B.6.3. RENAL FAILURE AND UREMIA

The occurrence of cataract appears to be higher in patients with renal failure.[119] Although the lens opacification may be transient when associated with hemodialysis in these patients and thought to be caused by the osmotic shock that dialysis causes, but Laqua (1972)[120] noted lens opacities before dialysis and suggested they were caused by uremia. Increased blood urea could lead to cataract in a similar way to that postulated in severe diarrhea.

After renal transplantation patients are treated with immunosuppressants usually including corticosteroids that may cause cataract. Posterior subcapsular lens opacities were observed in 19 out of 22 renal transplant recipients, aged 21 to 54 years in Hiroshima[121].

The case-control study in Edinburgh found that the mean urea level was significantly higher in the plasma of cataract patients compared with controls.[122] Diuretics may raise urea levels and thus contribute to these differences but when all diabetics and individuals receiving diuretics were excluded a relationship between high plasma urea and cataract remained.[123]

B.6.4. ENVIROMENTAL FACTORS: ULTRAVIOLET RADIATION

There is considerable international interest in the association between solar ultraviolet B radiation (UVB) and cataract. Much of this interest has resulted from concern about the health effects of the increasing levels of UVB reaching the earth's surface as a consequence of depletion of the stratospheric ozone layer.

Young suggests that sunlight is the primary causal factor in cataractogenesis, and strongly advocates the widespread distribution of sunglasses to prevent cataract.[124] Harding on the other hand suggests that sunlight is not a major etiological factor in human cataract formation.[125]

The lens is known to absorb UV-B and UV-A and change in lens clarity has been linked in animal experiments with short-term, high intensity exposure as well as chronic exposure to UV-B.

Epidemiologic studies have demonstrated cataracts to be more prevalent in sunny countries, such as Israel, than in cloudy countries, such as England.[126]

The Lens Opacity Case-Control Study[127] did not find an association between sun exposure and any type of cataract development. However, this study investigated only urban populations, and this may explain why no association was found. In both the Italian-American Cataract Study[128] and India-US Case-Control Study[129], sunlight exposure was associated with cataract formation. Taylor[130] studied 797

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watermen and went to great lengths to calculate an ultraviolet radiation exposure index on the basis of field variables such as outdoor hours worked, work location, and attenuation due to spectacle use and hat cover. He found a significant association between ultraviolet B radiation index and cortical cataract but found no association with other morphological cataract types.

Bochow et al.[131] studied the relationship between ultraviolet radiation exposure and posterior subcapsular cataracts. He not only discovered a significant association but also a dose-response relationship.

Schein et al.[132] studied the distribution of cortical opacities by lens quadrant in a prospective study of Chesapeake Bay watermen. The prevalence of cortical lens opacities increased with age, with a high degree of concordance between eyes. The inferonasal lens quadrant was the most common location involved both for new cataract development and for progression of pre-existing cataracts. Cataract formation in this quadrant was presumed to be most consistent with ultraviolet radiation damage on the basis of greater exposure in this area of the lens.

B.7. DRUG RELATED FACTORS

A number of commonly used medicinal agents have been implicated in lens opacification. Corticosteroids are the most important class of agents in this regard and are most commonly implicated in serious iatrogenically induced lens opacification.

B.7.1. CORTICOSTEROIDS

Since 1948 when cortisone was synthesized for the first time corticosteroids have had an enormous impact on medicine. It is estimated that between 10-60% of patients using systemic corticosteroids develop cataract, especially of the posterior subcapsular (PSC) type (Figure 3).[133]

Several mechanisms have been proposed in which corticosteroids may induce cataract formation including:

1. elevation of glucose levels;
2. inhibition of Na, K-ATPase;
3. increased cation permeability specifically in Berger's space;
4. inhibition of glucose-6-dehydrogenase;
5. inhibition of RNA-synthesis;
6. loss of ATP and
7. covalent bonding of steroids to lens proteins.[134]

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Figure 3[135]: Posterior subcapsular cataract

Posterior subcapsular cataract (PSC) is the hallmark of steroid cataracts. PSC opacities occur frequently with high doses (more than 15 mg prednisone or equivalent per day) and prolonged use (more than one year) of corticosteroids. Clinical trials have shown that PSC opacities secondary to oral corticosteroids may develop within as short a time as four months.[136]

Recent studies have suggested that the use of inhaled corticosteroids may perhaps be a significant risk factor for the development of cataract, maybe even more so than the use of oral corticosteroids.[137] These studies have again confirmed the importance of the "first-order effect".[138]

B.7.2. ALLOPURINOL

Allopurinol is an antihyperuricemic drug widely used for the treatment of hyperuricemia and chronic gout. In 1982, Fraunfelder et al[139] reported 30 cases of cortical and subcapsular cataracts associated with long-term use of allopurinol reported to the National Registry of Drug-Induced Ocular Side Effects (Oregon Health Sciences University, Portland). The observed lens changes appeared to have the characteristics of early age-related cataracts. At about the same time, Lerman et al[140,141] used phosphorescence spectroscopy to demonstrate in vitro the probable presence of allopurinol in cataractous lenses that had been extracted from patients treated with allopurinol.

Evidence from epidemiologic studies on the possible cataractogenic effects of allopurinol is, however, inconclusive. Two separate epidemiologic studies did not show an increased risk.[142,143] Garbe et al, on the other hand, using data from the Quebec universal health program, established that a clear relationship exists between the long-term administration of allopurinol and an increased risk for cataract extraction.[144]

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B.7.3. PHENOTHIAZINES

Epidemiologic research on the role of phenothiazines in cataractogenesis is limited. However, in 1965 the occurrence of ocular pigmentation and lens opacification in patients on high dose phenothiazine drugs, particularly chlorpromazine, was reported in several papers.[145,146] Phenothiazine is thought to combine with melanin and form a photosensitive product and this process may accelerate any predisposition to lens opacification from environmental insults such as solar radiation. A study involving schizophrenic patients showed an association between severity and grade of lenticular pigmentation and equivalent dose of phenothiazine intake.[147]

B.7.4. DIURETICS AND ANTIHYPERTENSIVES

The role of diuretics and antihypertensives in cataractogenesis is controversial. Harding and van Heyningen[148] reported that thiazide diuretics were used less frequently by patients who underwent cataract surgery than control subjects. More recently, the Beaver Dam Eye Study found that use of thiazides was associated with lower prevalence of nuclear cataract but increased prevalence of posterior subcapsular cataract.[149] Several other studies have found that use of diuretics was associated with increased risk of cataract.[150,151]

The calcium channel blocker nifedipine has been associated with increased risk of cataract extraction[152] and angiotensin-converting enzyme inhibitors with decreased risk of nuclear cataract.

B.7.5. ANTIMALARIAL DRUGS

Most drugs used in the treatment of malaria produce phototoxic side effects in both the skin and the eye. Cataract formation being one. The mechanism for these reactions in humans is unknown. A number of studies have been published that suggest a strong relationship between chloroquine use and cataract formation.[153,154] The basis of the relationship seems to lie in the photoxicity of chloroquine and related drugs.

Because malaria is a disease most prevalent in regions of high light intensity, protective measures (clothing, sun block, sunglasses or eye wraps) should be recommended whilst taking antimalarial drugs.

B.7.6. AMIODARONE

Amiodarone is a benzofurane derivative used for cardiac rhythm abnormalities. Its use is commonly associated with an asymptomatic keratopathy.[155] The anti-arrhythmic drug also produces anterior subcapsular lens opacities that are usually asymptomatic but progressive not affecting Snellen visual acuities but leading to subtle visual impairment as measured by contrast sensitivity.[156]

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B.7.7. HYPOCHOLESTEROLEMIC DRUGS

Cataracts in animals and man are in some instances associated with both genetic defects in enzymes that regulate cholesterol metabolism and the use of drugs that inhibit lens cholesterol biosynthesis.[157] The basis of this relationship apparently lies in the need of the lens to satisfy its sustained requirement for cholesterol by on-site synthesis, and impairment of this synthesis can lead to alteration of lens membrane structure.[158] Questions about the ocular safety of drugs, which can inhibit lens cholesterol biosynthesis, persist.[159] Concern over potential damage to the lens from the use of hypocholesterolemic drugs stems from the reports in 1962 by Kirby et al[160] and Laughlin et al[161]. Those studies showed that the treatment of patients with triparanol to lower blood cholesterol was associated with development of cataract. Drugs used to lower blood cholesterol are among the most widely prescribed medicines. One drug in the group, lovastatin is the third most prescribed drug in the United States.[162] This drug inhibits cholesterol synthesis in the lens and produces cataracts in canines[163]. Whether these drugs inhibit cholesterol biosynthesis in human lenses at therapeutic doses is yet unknown.

In one clinical trial a significant increase in cortical opacities was observed with the use of lovastatin.[164]

Hypolipidemic drugs are intended for life-long use and patients as young as 18 years can receive these drugs.[165] Thus, the consequences of inhibiting lens growth due to block of cholesterol biosynthesis may be difficult to assess in only a 1 – 5 year period. Long-term ocular safety of the vastatin drugs should perhaps be viewed in intervals of 10 – 20 years. The question of whether the vastatin drugs inhibit lens cholesterol biosynthesis in humans treated with standard therapeutic doses remains unanswered. Since very low concentrations of lovastatin and simvastatin are required to inhibit cholesterol synthesis in the lenses of animals (3 – 22 nM),[166]' and only five times the therapeutic dose of lovastatin decreased cholesterol accumulation by the rat lens, it at least appears possible that therapeutic doses could inhibit lens cholesterol biosynthesis in humans.[167]

C. CONCLUSION

Human lenticular opacification is **etiologically multifactorial.** However, mounting evidence in this field would seem to support the view that **lens opacification**, with its sequel of cataractogenesis, is **a preventable condition**.

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CHAPTER 3

DYSLIPIDEMIA AND LIPID PHYSIOLOGY - A BRIEF REVIEW

This dissertation describes, inter alia, the role of cholesterol in lens membrane structure, the source of this essential molecule and the effect of abnormal serum lipid parameters (i.e. LDL-, HDL, Total-Cholesterol and Triglycerides) on human lenticular opacification. In order to appreciate this topic better, a short précis of human lipid metabolism, in particular cholesterol is given in order to understand the relationship between lipids and lenticular opacification.

A. INTRODUCTION

Lens opacification[1] and cardiovascular disease[2] are two of the main causes of morbidity worldwide. The relationship between cholesterol and cardiovascular heart disease is well documented [3,4]. The relationship between cholesterol and lens opacity is, however, far less well appreciated.

The lens is unique in that it contains a relative abundance of cholesterol in the fiber cell plasma membrane[11] and furnishes its needs for the latter by onsite biosynthesis[5].

Dyslipidemia can be defined as abnormal serum lipid levels containing one or more of the following elements:

- Raised total serum cholesterol
- Raised serum triglicerides
- Reduced serum HDL-Cholesterol
- LDL:HDL ratio >5

B. SYSTEMIC LIPID PHYSIOLOGY

SERUM LIPIDS

Lipids in the body have both structural and nutritional function and are represented by wide variety of molecules: triglicerides, phospholipids, cholesterol etc. The disposition of lipids after absorption is of critical importance since elevated serum lipid levels are associated with a variety of disease processes. The transport and fate of dietary lipids are schematically summarized in Figure 1.

Lipids are biomolecules made up of carbon, hydrogen, and oxygen. Because of their nonpolar structure, they are not very soluble in water. Lipids are the most diverse group of biomolecules. In addition to the true lipids,

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this category includes three lipid-related substances: phospholipids, steroids and eicosanoids. True lipids contain a simple 3-carbon molecule known as **glycerol** and long carbon-chain molecules known as **fatty acids.** Fatty acids link to glycerol to form mono-, di-, or triglycerides. Triglycerides are the most important form of lipid in the body: More than 90% of our lipids are in this form. Phospholipids are diglyserides with a phosphate group attached to the single carbon that lacks a fatty acid. Phospholipids are important components of cell membranes.

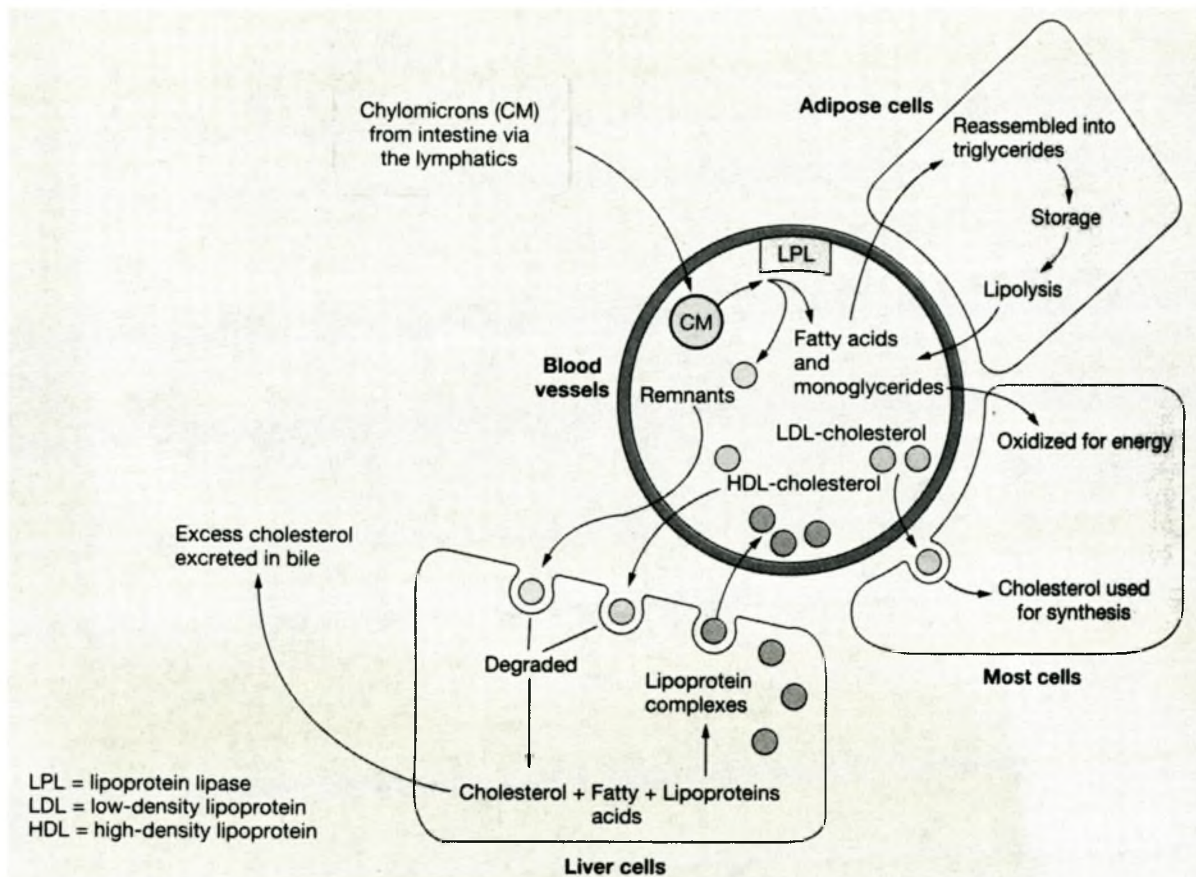

Figure 1[6]. Transport and fate of dietary fats

Complexed or packaged lipids absorbed from the gut take the form of chylomicrons (CM's) which gain access to the liver. In the liver the chylomicrons are degraded into their constituent components. These liberated lipid components are then once again complexed with proteins to form VLDL and HDL. The greater the percentage of protein in the package the higher the density of the package and the greater the water solubility thereof. HDLs are desirable whilst LDLs are thought to sediment in vascular walls impeding flow, damaging the intima and provoking a destructive inflammatory response.

LDL is sometimes called the "bad cholesterol" because elevated levels are associated with atherosclerosis. The second most common lipoprotein is HDL, also known as the "good cholesterol" because HDL takes cholesterol into the liver cells, where it is metabolized or excreted. (*L* also stands for *Lethal* and *H* for *Healthy!*). The ratio of the HDL-cholesterol to LDL-cholesterol in the plasma is as important as the absolute concentration.

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The protein components of the lipoproteins are called **apoproteins**, or **apolipoproteins**. LDL complexes contain apoprotein B (apo B), a molecule that combines with specific receptors to allow cholesterol and triglycerides to be endocytosed into most cells of the body. HDL complexes contain apo E, which activates receptors in the liver. Several inherited forms of hypercholesterolemia have been linked to defective forms of either apo E or apo B.

As alluded to above, the major plasma lipids, including cholesterol (or total cholesterol) and the triglycerides (TG), do not circulate freely in solution in plasma, but are bound to proteins and transported as macromolecular complexes called lipoproteins[7].

The major lipoprotein classes are:

- Chylomicrons,
- Very Low Density (pre-ß) Lipoproteins (**VLDL**),
- Low Density (ß-) Lipoproteins (**LDL**), and
- High density (α-) Lipoproteins (**HDL**);

Hyperlipidemia may result either from:

- Overproduction of VLDL, or
- Defective clearance of VLDL, or
- Increased conversion of VLDL to LDL.

Overproduction of VLDL by the liver may be caused by[8]:

- Obesity,
- Diabetes mellitus,
- Alcohol excess,
- Nephrotic syndrome, or
- Genetic disorders.

When dietary cholesterol (as a constituent of chylomicron remnants) reaches the liver, the resulting elevated levels of intracellular cholesterol suppress LDL-receptor synthesis[9]. The reduction in the *number* of receptors result in higher levels of plasma LDL and therefore of TG. Saturated fatty acids also increase plasma LDL and TG levels due to reduced *activity* of LDL receptors[10]. When dietary cholesterol and saturated fatty acid intake is high, they are thought to account for an average increase of between 0.65 and 1.03 mmol/L of LDL blood levels—enough to increase the risk of coronary artery disease (CAD) significantly [11].

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C. MOLECULAR BIOCHEMISTRY OF CHOLESTEROL

SYNTHESIS

Cells obtain cholesterol by *de novo* synthesis or through uptake from circulating lipoproteins[12]. The rate-limiting step in cholesterol biosynthesis is the reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) to mevalonic acid, which in turn is controlled by the activity of HMG CoA reductase[13]. Rates of cholesterol biosynthesis in cells are tightly controlled by feedback regulation of HMG CoA reductase.

FUNCTION

MEMBRANE-PROTEIN FUNCTIONS

One important function of cholesterol is to modulate the activity of membrane-bound proteins as well as the permeability of membranes to solutes. Cholesterol can seal membranes against the non-specific passive leakage of small molecules[14]. Membrane Na^+ - Ca^{2+} exchanger and (Na^+ + K^+) ATPase activities of cardiac sarcolemma are highly dependent on the presence of cholesterol[15]. These cholesterol effects appear to be due to a direct interaction of cholesterol with functional membrane proteins.

MEMBRANE-LIPID FLUIDITY (ORDER)

In addition to direct effects on proteins, cholesterol can regulate membrane activities by modulating membrane fluidity or structure. In general, increasing the cholesterol content of membranes decreases transport of ions and organic solutes[16]. Modulation of membrane fluidity by cholesterol depends on:

- The relative abundance of cholesterol-rich versus cholesterol-poor domains,
- The phospholipid composition of the membrane and
- The hydrocarbon chain composition of the phospholipids.

Although most studies show that adding cholesterol to natural and artificial membranes increases membrane order [17], some studies report that cholesterol can disrupt membrane lipid[18]. The high cholesterol content of lens membrane has been assumed to promote the structural rigidly of this membrane[19].

BASIC LENS LIPID PHYSIOLOGY

Since the lens grows throughout life by the terminal differentiation of epithelial cells, 10 microns in height, into enormously elongated fiber cells, up to 12 mm in length[20], there is a continuous need for cholesterol to sustain

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fiber cell membrane formation. Cholesterol is also needed for membrane formation by proliferative lens epithelial cells in order for them to proceed through the cell cycle[21].

SYNTHESIS AS SOURCE

The very avascular nature of the lens suggests that the lens derives its cholesterol from *de novo* synthesis. The lens is indeed able to obtain its cholesterol by biosynthesis. Initially it was assumed that the lens, like other tissues, could obtain some cholesterol from circulating lipoproteins[22,23]. In later work the cholesterol found in aqueous humor (1 g/ml) was present in high-density lipoprotein fraction[24]. The life-long requirement of the human lens for cholesterol was confirmed when it was showed that the human lens accumulates cholesterol at a constant rate from an early age right through to old age.[25]

REGULATION OF SYNTHESIS

The activity of HMG CoA reductase appears to control the rate of cholesterol synthesis in the lens, as in other tissues. Regulation of lens cholesterol synthesis is closely linked to the cholesterol required to sustain the massive membrane formation that accompanies fiber cell elongation.

CHOLESTEROL CONCENTRATION IN LENS TISSUE

Cholesterol plays an important role in the biology of the ocular lens because altering the composition and content of lens sterols can be associated with cataract formation. However, consideration of the cholesterol concentration in total lens mass alone does not suggest special significance. Based on whole lens-wet weight, the cholesterol concentration is modest, being about 4 – 8 mg cholesterol/g in the human[26], 1 – 2 mg/g in rat[27], and 1-2 mg/g in the bovine lens[28]. However, essentially all of the cholesterol is confined to a small compartment of the lens total volume, the fiber cell plasma membrane. Plasma membrane occupies only about 1% of lens total volume[29] yet, 85 – 90% of the total lenslipid precipitated upon centrifugation of homogenates of whole human lenses is from this site of origin[30].

CHOLESTEROL-PHOSPHOLIPID RATIO

Although the total concentration of cholesterol in the lens is unremarkable, its relative abundance in the fiber cell plasma membrane is uniquely high. Plasma membrane of most animal cells typically contain 0.5 – 1.0 molecules of cholesterol to 1.0 molecule of phospholipid[31]. The cholesterol to molar ratios in membrane of rat, chicken, cow and human lens range from 1 – 4[32] with nuclear membrane having the higher ratios[33]. This ratio increases with age and may feature as a factor in age-related cataractogenesis.

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MEMBRANE DISTRIBUTION

a) MEMBRANE FRACTIONS

Little is known about the distribution of cholesterol among different fractions of lens fiber cell plasma membrane. Lens membrane, and especially cortical membrane, contain abundant junctional domains which appear similar to gap junctions'[34]. These fiber junctions are believed to communicate with adjacent cells and provide routes for passage of small molecules from superficial to deeper lying cells[35]. There appears to be little difference in the distribution of cholesterol between junctional and nonjunctional domains of lens membranes.[36]. The monolayer of epithelial cells located on the lens' anterior surface is another source of plasma membrane. No specific information on the lipids of its plasma membrane could be found.

b) MEMBRANE BILAYER

Within the membrane lipid bilayer, cholesterol may exist in patches. Li et al[37] estimated that phospholipids occupy less than one-third of the surface of human lens nuclear membrane and suggested that this membrane is organized into mosaics of phospholipid bilayer and cholesterol patches. This suggestion is consistent with the well-established view that the lateral distribution of cholesterol in membranes is highly asymmetrical; i.e., plasma membrane contains cholesterol-rich and -poor domains.

FUNCTION OF CHOLESTEROL IN LENS TISSUE

MEMBRANE-LIPID FLUIDITY (ORDER): LENS

The role of cholesterol in the lens is to maintain cortical and nuclear membrane lipids at similar levels of fluidity. This could be important for lens optical clarity and can be achieved only by having a much higher cholesterol content in nuclear than cortical membrane. The driving force for the high relative concentration of cholesterol in the nuclear membrane is the loss of phosphoglycerides with aging of the fiber cell[36].

CLINICAL SIGNIFICANCE

Clinical observation, based upon the patho-physiology described above, has lead us to suspect that a proper look at the association of dyslipidemia and lenticular opacities is warranted as this has, to our knowledge, not been done before. Such a study was therefore undertaken and its results form the substance of chapters 4 and 5 of this thesis.

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CHAPTER 4

CORTICAL LENS OPACITIES IN DYSLIPIDEMIC PATIENTS

ABSTRACT

PURPOSE

To determine the characteristics and prevalence of lenticular opacification in patients with underlying dyslipidemia.

METHODS

Adult patients (n = 80) of both genders and irrespective of age were enrolled in the trial if they met the inclusion criteria for dyslipidemia. Patients were included if their fasting serum cholesterol and triglyceride concentrations were > 5.2 mmol/l and >2.3 mmol/l, respectively when measured on three separate occasions over a one month period. Patients were excluded if they suffered from any condition known to cause, or predispose, to elevated lipid levels or lens opacification. Lenticular changes were assessed by means of a slit-lamp through the fully dilated pupil, and other physical signs were documented subsequent to thorough physical evaluation.

RESULTS

In addition to the classical clinical signs of dyslipidemia, 31% of patients had cortical lens opacities. Cortical opacities were twice as prevalent as achilles tendon thickening, 16.3% in our study, the second most prevalent sign of elevated lipid levels. In the subgroup of patients < 50 years, 55% had lenticular opacities, predominantly cortical (80%).

CONCLUSIONS

Cortical lens opacification was the most prevalent sign of dyslipidemia and it occurred at a relatively young age in our trial population in those patients who were affected. Cortical lenticular opacification should be regarded as an indication for blood lipid profile evaluation.

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A. INTRODUCTION

Jahn et al [1] attempted to determine the role of glucose and lipid metabolism in the formation of cataract in elderly individuals undergoing cataract extraction. They found that patients with posterior subcapsular cataract had higher concentrations of fasting serum triglycerides, and were significantly younger, than patients with nuclear or cortical cataract. Furthermore the results suggest that the association of hypertriglyceridemia, hyperglycemia and obesity, favors the formation of a specific morphologic type of lens opacity, i.e., posterior subcapsular cataract, occurring at an early age.

In order to assess the prevalence of lenticular opacities in patients with dyslipidemia (raised serum cholesterol and triglycerides) a group of 80 dyslipidemic patients were subjected to a general physical examination and an ophthalmic examination of the fully dilated eye

B. METHODS

Inorder to obtain a study group with maximum homogeneity, only patients meeting the appropriate criteria were enrolled.

INCLUSION CRITERIA

Patients were included, irrespective of gender, if they were older than 18, and younger than 90 years of age, and had:

elevated total serum cholesterol levels (>5.2 m mol / L);
low plasma HDL cholesterol levels (< 1.8 m mol / L);
high plasma triglyceride levels (> 2.3 m mol / L).

(Patients were included in the study only if their lipid profiles were found to be abnormal on three separate occasions over the course of a four-week period; blood samples taken after an overnight fast were evaluated in all instances.)

Patients were excluded if they were pregnant or lactating females or if they had:

severe hypertension (diastolic pressure > 115 mm Hg);
a history of cardiovascular disease;
diabetes mellitus (fasting blood glucose >7.8 m mol / L);
hypothyroidism (TSH < 7.5 mU / L);
a history of a neoplasm (past or present);
renal impairment (serum creatinine > 170 micro-mol / L);

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pancreatitis;
disease of the gall bladder, including cholelithiasis;
a history of severe gastro-intestinal disease;
a positive HIV test.

All classical physical signs of abnormal lipid variables were documented, namely

- **Xanthomas:**
  - On the Achilles tendon
  - On the hands
  - On the elbows
  - On the knees
- **Palmar yellow striae**
- **Xanthelasma**
- **Corneal arcus.**

Lenticular opacities were classified as being cortical, nuclear or subcapsular on the basis of the characteristics and grading as indicated here below:

1. Cortical opacities

- Water clefts, vacuoles, and flakes - none, few, moderate, or many.
- Wedges and spokes – involving 1,2,3 or 4 quadrants.
- Maximal inward extension - minimal, moderate or advanced.

2. Nuclear opacities

- Tissue discoloration – normal colour, pale yellow, yellow, dark yellow or brown.

3. Subcapsular opacities

- Posterior capsule involvement –graded 1-4.
- Anterior capsule involvement – graded 1-4.

Both a specialist physician and an ophthalmologist examined all the patients.

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C. RESULTS

DEMOGRAPHIC DATA

Eighty patients were analyzed and Table 1 reflects their demographic data.

Table 1: Demographic data

Variable	Mean	Standard deviation
Age	53.9y	11.8y
Blood pressure		
Systolic	134mmHg	18mmHg
Diastolic	84 mmHg	9mmHg
Body Mass Index	28.29 kg/m^2	4.82kg/m^2
	Number	
Race		
Mixed race	15 (18.8%)	-
Caucasians	65 (81.3%)	-
Gender		
Male	48 (60%)	-
Female	32 (40%)	-
Smokers		
Present	23 (28.75%)	-
Past	34 (42.5%)	-
Never	23 (28.75%)	-
Alcohol consumers	55 (68.8%)	-

The study group was predominantly male, caucasian and smokers (past and present). Most patients – 68.8% - admitted regular alcohol consumption. The mean systolic and diastolic blood pressure data, 134 ± 18 and 84 ± 9mmHg, respectively, fell within the normal range for age. The body mass index (BMI) of the group was significantly greater than the norm (i.e. 28.89 ± 4.82 kg/m^2).

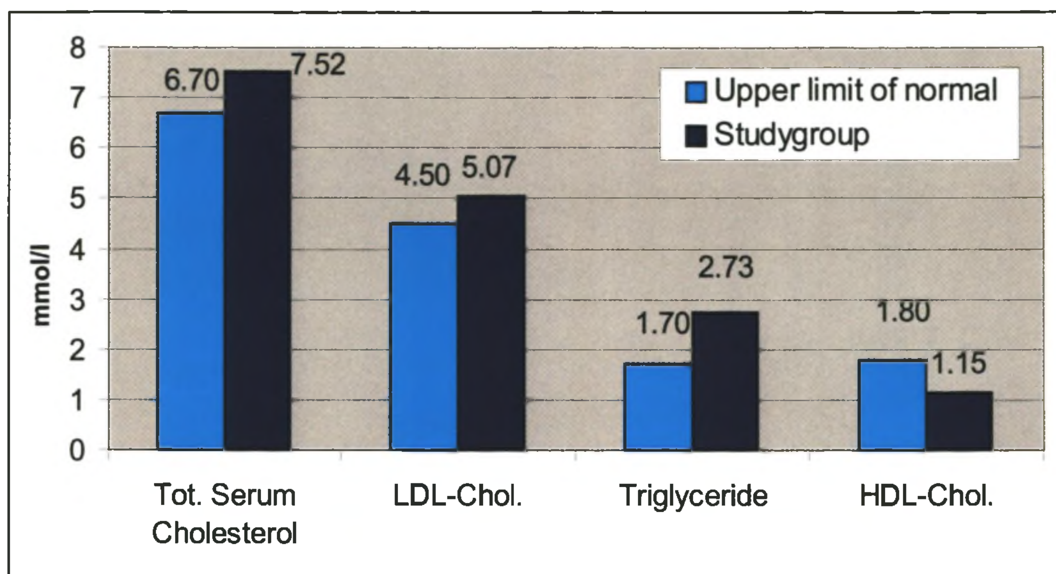

Figure 1: Mean serum lipid parameters of the study group (n=80) in comparison to the upper limit of normal (mmol/l).

Figure 1 depicts the serum lipid variables. Patients were included in the study only if total serum cholesterol was > 5.2 mmol/l, when measured on three separate occasions over a four week period, and LDL and HDL were uniformly > 2.3 mmol/l and < 1.8 mmol/l, respectively.

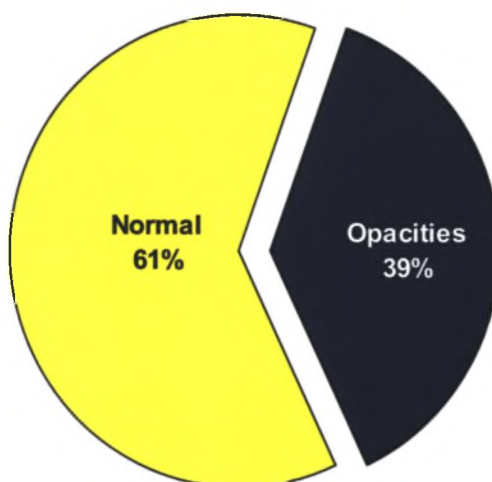

Figure 2: Prevalence of lens opacities in the study group.

The prevalence of lenticular opacities divided the studygroup into two cohorts i.e. those with normal lenses (61%) and those with opacities (39%).

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SUBGROUP DATA

Table 2: Comparison of the subgroup with normal lenses (61%) to the subgroup with lenticular opacities (39%).

Variable	Normal Mean (SD)	Opacity Mean (SD)
Age	55.3y (10.9) y	54.7 (14.8) y
Blood pressure		
Systolic	135.6 (21.19) mmHg	140.0 (21.81) mmHg
Diastolic	84.4 (10.03) mmHg	86.1 (9.83) mmHg
Body mass index	28.55 (4.84) kg/m^2	27.81 (4.84) kg/m^2
Total Cholesterol	7.3 (2.01) mmol/l	7.91 (2.00) mmol/l
LDL-Cholesterol	5.05 (1.54) mmol/l	5.11 (1.46) mmol/l
HDL-Cholesterol	1.16 (0.39) mmol/l	1.14 (0.31) mmol/l
Triglycerides	2.64 (1.89) mmol/l	2.89 (1.02) mmol/l
Uric Acid	3.64 (0.91) mmol/l	3.98 (1.25) mmol/l

Comparative analysis in respect of age, blood pressure, body mass index, lipid index and uric acid profile supported the concordance between the two subgroups. (Table 2)

Table 3: Age distribution of patients with opacities compared to other population based studies.

Age group (years)	Percentage of opacities		
	Studygroup	BES[2]	BDES[3]
30-40	33.33	N/A	N/A
40-50	50.00	4.7	8.3
50-60	18.51	24.5	26.5
60-70	33.33	57.5	56.7
70-80	66.67	85.9	70.5
80+	33.33	98.3	N/A

BES: Barbados Eye Study.

BDES: Beaver Dam Eye Study.

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N/A: Not available.

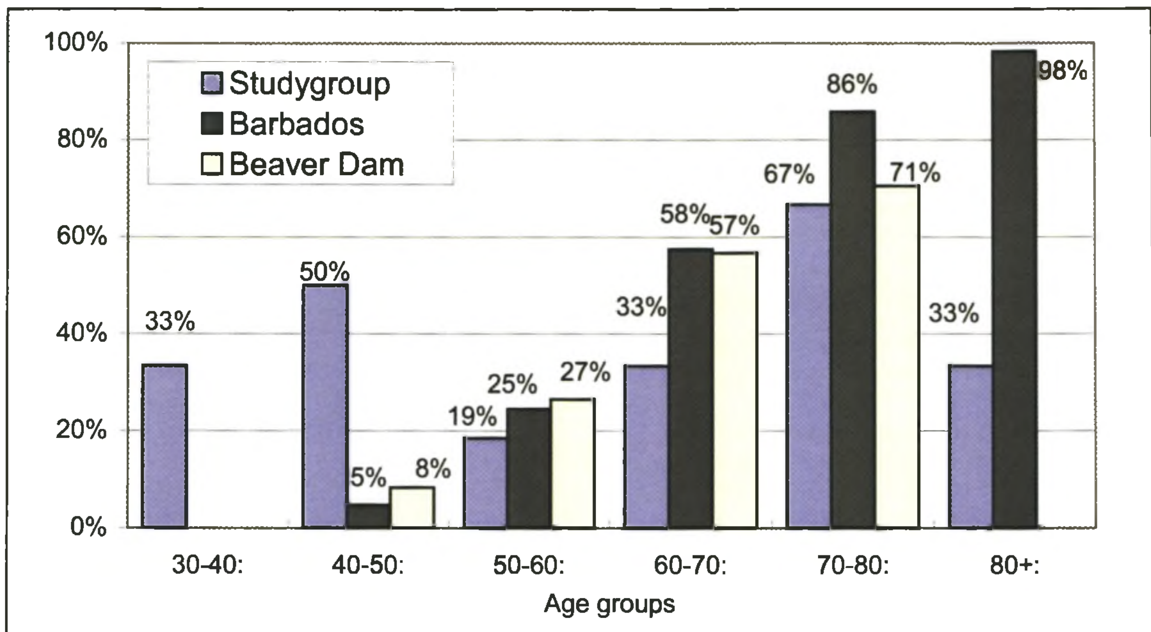
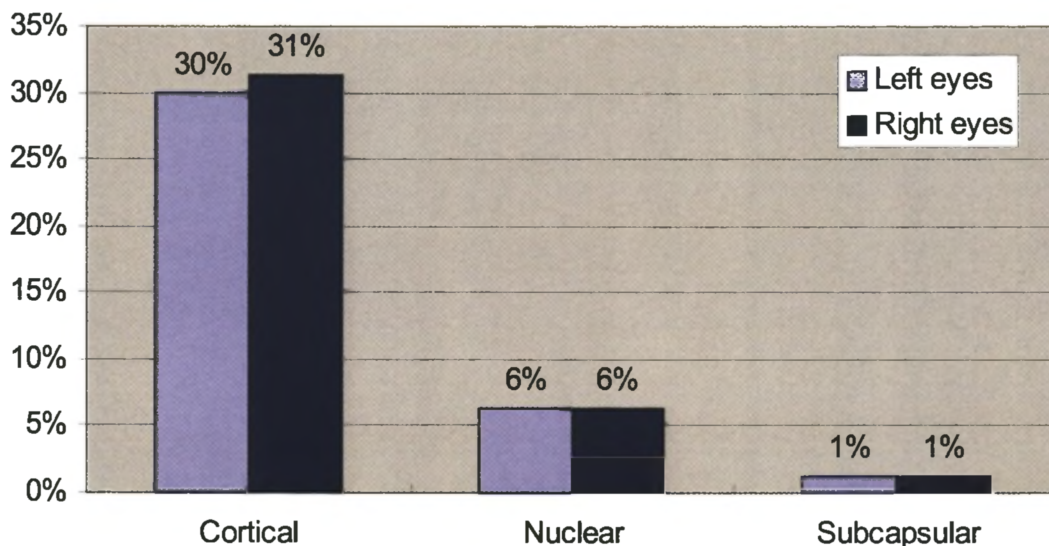


Figure 3: Studygroup comparisons

The prevalence of lenticular opacity in dyslipidemic patients in the age group 30 – 40 years was 33%. This age group was not studied in the Barbados Eye Study (BES) or in The Beaver Dam Eye Study (BDES) and consequently data for comparison are not available. In the 40 – 50 year age group the prevalence of lenticular opacities in the study patients was 50% compared to 4.7% in the BES and 8.3% in BDES. Differences in the older age groups were not prominent (Figure 3).

Figure 4. Types of opacities compared to laterality



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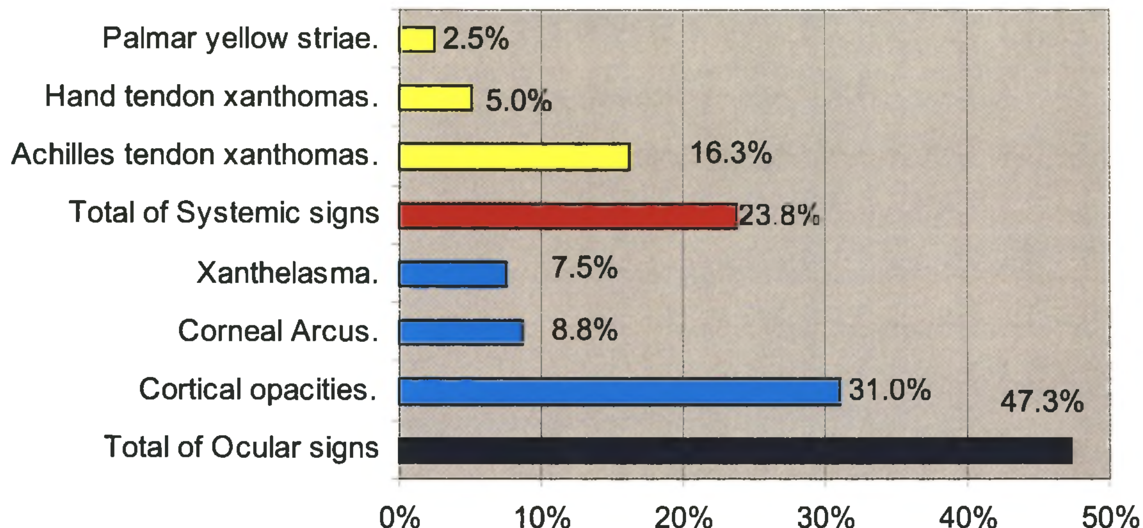
No lateralising bias was observed in the subgroup with lenticular opacities (Figure 4). This is supported by the findings in normal clinical practice, where a predilection for laterality of age related cataracts does not exist.

Figure 5. Physical signs associated with dyslipidemia.



The distribution of dyslipidemia related signs in the study group was as follows:

- Xanthelasma 7.5%
- Corneal arcus 8.8%
- Achilles tendon involvement 16.3%.
- Cortical lens opacities 31.0%

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D. DISCUSSION

Modern medicine today aspires to the early detection of disease processes with the aim of early intervention in an attempt either to halt the progression or to reverse the process.

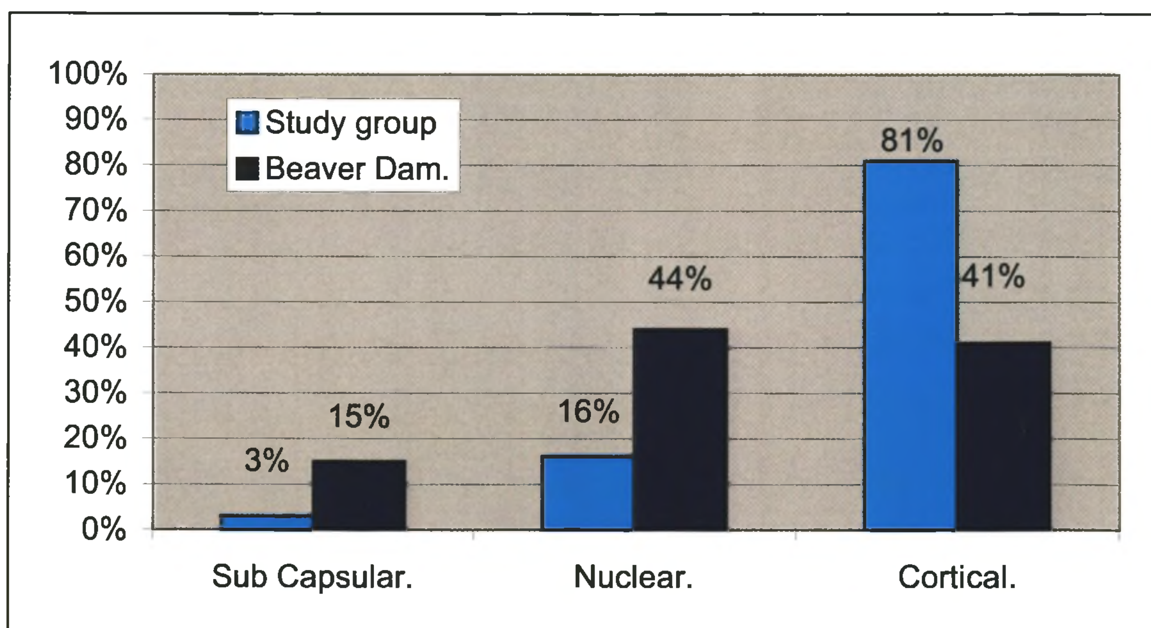
Although the classic systemic signs of dyslipidemia are well appreciated, i.e. Xanthomata, xanthelasma, thickening of the achilles tendon and corneal arcus, in this study the prevalence of one or more of the ocular signs was far greater than that of the systemic signs, 23.8% for the former as opposed to 47.3% for the latter.

It is noteworthy that the most frequent ocular sign – cortical lenticular opacity – occurred twice as frequently as the most frequent systemic sign – achilles tendon thickening; Figure 5 refers.

Although a control group of patients was not evaluated simultaneously, valid comparisons could be made between our data and that of other population-based studies. Two highly regarded studies, both comprising large populations were used as the basis of comparison. The one study was effected in a population comprised almost exclusively of whites (the Beaver Dam Eye Study[36], BDES, Wisconsin, USA, n = 4926), whilst the other study was effected in a population comprised predominantly of blacks (Barbados Eye Study[35], BES, Barbados, n = 4709). Our study was effected in a population comprised predominantly of whites (81.3%), the complement all being patients of mixed race. In the BDES and BES, 40% and 41% of patients, respectively, were found to have lens opacities and cataract, and this is entirely consistent with the prevalence (39%) found in our study-group comprised of patients with dyslipidemia. Although the prevalence of lens opacification/cataract is comparable in the respective populations-BDES, BES, and our study group- there were significant differences between the groups in respect of the age distribution of the younger (40-50 years) group (Figure 3 refers).

In Figure 6 the prevalence of the different morphological subtypes of opacities in our study and in the Beaver Dam Eye Study are compared. It is clear that cortical opacities occurred twice as frequently in the hyperlipidemic group as in the normal population whereas nuclear opacities occurred with more than twice the frequency in the normal population.

Figure 6: Prevalence of the morphological types of opacities in the study group vs. Beaver dam.



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The mean age of the patients was 53.9 ± 11.8 years and not surprisingly the prevalence of nuclear cataracts was relatively low. Since nuclear degeneration correlates strongly with age and aging it is more prevalent in the elderly. In contrast, cortical lens opacities were highly prevalent in this study group.

Patients with conditions known to induce lens opacification e.g., diabetes mellitus, neoplastic disease, hypothyroidism, pancreatitis, renal failure, severe hypertension and HIV/AIDS complex, among others, were meticulously excluded from the trial. Maritz[4] have shown that dyslipidemic patients have an increased risk of developing adult onset diabetes mellitus with advancing age than the general population. No patient in the study group had a fasting blood glucose concentration in excess of 7.8 mmol/l and consequently the contribution of hyperglycemia to the high prevalence of cortical opacification is highly unlikely.

The population of the BES study was comprised predominantly of black people and the comparative data from the trial appeared to reveal that black people are at greater risk of developing cortical opacities than caucasians and that the latter, in turn, are at greater risk of developing nuclear opacities.

In contrast to the BES study the patients in this study group were comprised predominantly of caucasians, i.e., 81.3%, the complement all being individuals of mixed race. It is possible that cortical opacities are equally prevalent in black and white people and the data provide sufficient motivation for further assessment of this factor in the etiology of lens opacification.

Defining a cataract is difficult. Harding defines it as "An opacification of the ocular lens sufficient to impair ocular vision"[5]. This dissertation deliberately steers away from the term cataract because most of the lens changes were not cataracts according to the Harding definition but rather lenticular opacities as described by the Lens Opacities Classification System II (LOCS II)[6]. None of the observed opacities were severe enough to cause substantial visual impairment.

It has been reported that corneal arcs represent a trustworthy sign of dyslipidemia only in patients less than 50 years age and that 60% of patients with periocular xanthelasma are normolipidemic[7].

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E. COMMENTS AND CONCLUSIONS

1. Dyslipidemic patients are more likely to develop cortical opacification than the normal population.
2. Cortical lens opacification manifests at a younger age than does nuclear opacification.
3. It is essential that an abnormal lipid profile be diagnosed or detected as early as possible in order to achieve the maximum possible benefit from therapeutic intervention.
4. Cortical lens opacification in the patient younger than 50 years of age should alert the ophthalmologist to arrange for diagnostic serum lipid assessment.
5. The young patient with dyslipidaemia should undergo regular slitlamp examination of the lens after full dilatation of the pupil in order to detect early signs of lens opacification.
6. Cortical lenticular opacification should be regarded as one of the most common, and hence reliable, clinical signs of dyslipidaemia.

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CHAPTER 5

SERUM LIPID COMPONENTS AS RISK FACTORS FOR LENS OPACITIES

ABSTRACT

AIM

To determine whether a correlation exists between the different plasma constituents of dyslipidemic subjects and the prevalence of human lenticular opacities

METHODS

Patients (n = 115) of both genders and irrespective of age were enrolled in the trial if they met the inclusion criteria for dyslipidemia.

Patients were included if their fasting serum cholesterol concentrations were > 5.2 mmol/l or their serum LDL:HDL ratios exceeded 5 when measured on three separate occasions over a one month period.

Patients were excluded if they suffered from any condition known to cause, or predispose them to, elevated lipid levels or lenticular opacification. Lenticular changes were assessed by means of a slit-lamp through the fully dilated pupil and other physical signs were documented subsequent to thorough physical evaluation.

RESULTS

An extremely strong correlation (p <0.0001) was found to exist between low HDL Cholesterol levels (i.e. hypoalphalipoproteinaemia) and the development of lens opacities. Below a HDL-C level of 1,5mmol/l subjects had a seven-fold higher calculated risk of falling in the lens opacity subgroup than those with HDL-C levels above 1,5mmol/l [ODDS RATIO=7.33 (95% CI = 2.06-26.10, p trend=0.001)]. An equally strong correlation existed between high (>5) LDL:HDL ratios and the development of lens opacities (p<0.0003). The odds ratio for falling into the cataract subgroup if the individuals LDL:HDL ratio exceeded 5 was 2.35 (95%CI=1.09-5.04, p trend=0.014). No statistically significant correlations between the other serum lipid parameters or patient age and opacification could be established.

CONCLUSIONS

Low levels of HDL-Cholesterol together with high LDL:HDL ratios have often been implicated in the development of aterosclerotic heart disease. This study strongly suggests that a correlation also exists between the same two risk factors and the development of adult lens opacification.

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A. INTRODUCTION

The first part of the study (chapter 4) concluded that dyslipidemic patients develop cortical lens opacities more frequently and at an earlier age than the normal population, and that cortical lens opacities should be regarded as one of the most common, and hence reliable, clinical signs of dyslipidemia.

The next logical question that arose was whether total serum cholesterol or any of the different fractions that make up the lipogram (i.e. LDL-Cholesterol, HDL-Cholesterol, Trigliserides, LDL:HDL ratio) could be associated with the phenomenon of cortical lens opacification?

This next study was therefore designed to examine this question.

B. METHODS

The previous group of 80 subjects was expanded to include 115 subjects. These were all individuals with proven dyslipidemia and were subjected to both a general physical examination by a specialist physician and a slitlamp ophthalmic examination of the fully dilated eye by an ophthalmologist.

In order to obtain a study group with maximum homogeneity only patients meeting the following criteria were enrolled:

<u>INCLUSION CRITERIA:</u>

- Male or female (18-90 years of age)
- High serum Total Cholesterol (>5.2mmol/l)
- LDL:HDL ratio >5.

<u>`EXCLUSION CRITERIA:</u>

- Pregnant or lactating females
- Severe hypertension (diastolic blood pressure >115 mmHg)
- History of cardiovascular disease
- Diabetes mellitus (fasting blood glucose >7.8mmol/l)
- Hypothyroidism defined as TSH >7.5mU/l
- Any malignant tumor
- Significant renal impairment (serum creatinine >170µmoll/l)
- History of pancreatitis
- Patient with gallbladder disease including cholelithiasis
- History of gastro-intestinal disease
- HIV antibody positive.

Fasting blood samples were obtained from each individual on three occasions over a period of 4 weeks. Patients were only included in the study if their lipid variables adhered to the inclusion criteria on each of the three visits.

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All classical physical signs of abnormal lipid variables were documented, namely

- **Xanthomas:**
  - On the Achilles tendon
  - On the hands
  - On the elbows
  - On the knees
- **Palmar yellow striae**
- **Xanthelasma**
- **Corneal arcus.**

Lenticular opacities were classified as being cortical, nuclear or subcapsular on the basis of the characteristics and grading as indicated here below:

I. Cortical opacities

- Water clefts, vacuoles, and flakes - none, few, moderate, or many.
- Wedges and spokes – involving 1,2,3 or 4 quadrants.
- Maximal inward extension - minimal, moderate or advanced.

2. Nuclear opacities

- Tissue discoloration – normal colour, pale yellow, yellow, dark yellow or brown.

1. Subcapsular opacities

- Posterior capsule involvement –graded 1-4.
- Anterior capsule involvement – graded 1-4.

Both a specialist physician and an ophthalmologist examined all the patients.

STATISTICAL ANALYSIS

All stastics were generated using the Statistica™ 1984-2000 (Release 5.5) by StatSoft, Inc, USA.

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C. RESULTS

DATA PROCESSING

The data presented in this section have been processed to support the following:

I. Demographic data of the studygroup together with the serum lipid profile. (Table 1 & 2, Figure 1)

II. The prevalence of lens opacity subtypes. (Figure 2)

III. Comparative HDL-Cholesterol data between the two subgroups, including intra-group stratification. (Figure 3 & 4)

IV. Comparative LDL:HDL ratio data between the two subgroups. (Figure 5 & 6)

I. DEMOGRAPHIC DATA AND SERUM LIPID PROFILE

The studygroup consisted of 115 predominantly white [94/115(82%)] subjects. The rest of the group (21/115 or 18%) were of mixed race decent. Gender distribution was 74(64%) male and 41(36%) female. The group was also relatively young with the mean age 49.1 years (SD=10.2). (Table 1)

Table 1. Demographic data of the studygroup (n=115)

Variable	Mean	Standard deviation
Age	49.1y	±10.2y
	Number	
Race		
Mixed race	21 (18%)	-
Caucasians	94 (82%)	-
Gender		
Male	74 (64%)	-
Female	41 (36%)	-

Table 2 and Figure 1 depicts the mean values of the different lipogram components in the studygroup (n=115).

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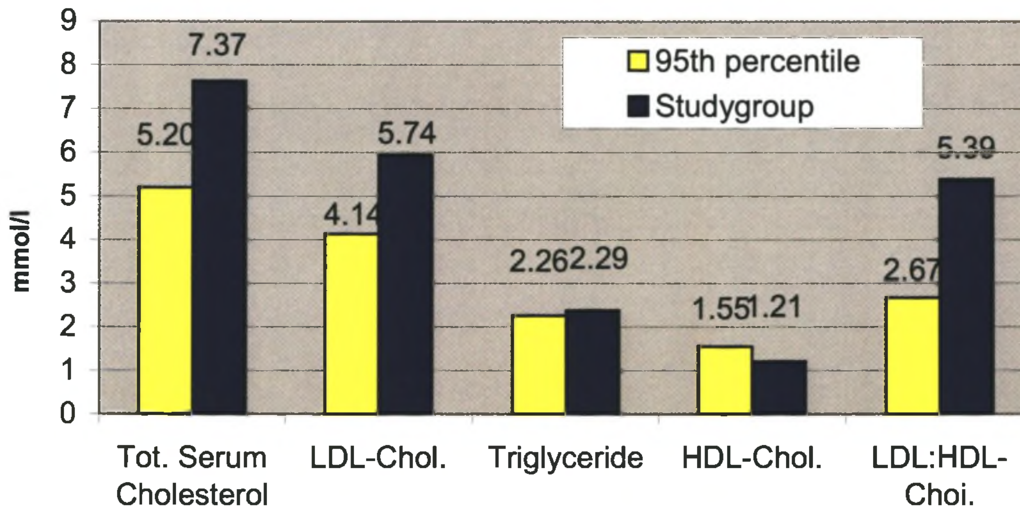
Table 2. Mean age and serum lipid profiles of the subgroup with clear lenses (n=68) and the subgroup with lens opacities (n=47).

Variable	Clear Lens Subgroup Mean (± SD)	Lens Opacity subgroup Mean (± SD)
Age	47.3y ± 9.8y	50.9y ± 10.6
Total Cholesterol	7.33 ± 1.90 mmol/l	7.46 ± 1.82 mmol/l
LDL-Cholesterol	5.65 ± 1.89 mmol/l	5.84 ± 1.75 mmol/l
HDL-Cholesterol	1.35 ± 0.35 mmol/l	1.02 ± 0.27 mmol/l
Triglycerides	2.27 ± 1.75 mmol/l	2.34 ± 1.05 mmol/l

Figure 1. Serum lipid parameters of the study group (n=115) compared to the upper limit of normal i.e. 95th percentile (mmol/l).



The mean Total serum Cholesterol, Triglyceride and LDL-Cholesterol levels exceeded the 95th percentile of normal whereas the mean serum HDL-Cholesterol was lower than the 95th percentile of normal. The studygroup was divided into two cohorts, i.e. a subgroup with opacities (41%) and a subgroup with clear lenses (59%). Analysis of the two subgroups (Table 2) suggested:

no difference in the mean age of the two subgroups (p=0.07) and

no differences in the following lipid parameters of the two subgroups:

Total Serum Cholesterol (p=0.71)

Serum LDL-Cholesterol (p=0.55).

Serum Triglycerides (p=0.81)

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II. PREVALENCE OF LENS OPACITY SUBTYPES

The prevalence of lenticular opacities divided the studygroup into two cohorts i.e. those with normal lenses 68 (59%) and those with opacities 47 (41%). In the lens opacity group the majority [36 (77%)] presented with cortical opacities. The subgroup with opacities included all opacities [nuclear 9(19%), cortical 36 (77%), and posterior subcapsular 2 (4%)]. (Figure 2)

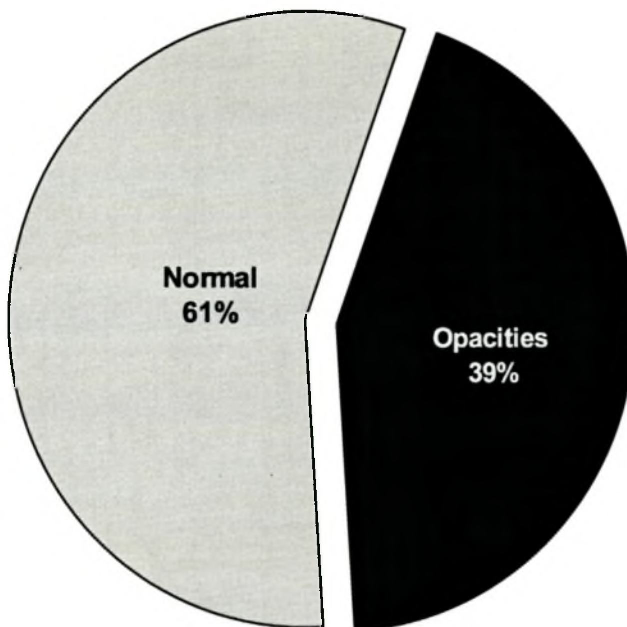

Figure 2. Prevalence of lens opacities(anatomical subtypes) in the studygroup.

III. COMPARISON OF HDL-CHOLESTEROL LEVELS BETWEEN THE TWO SUBGROUPS

The HDL-Cholesterol levels of the two subgroups differed. The mean HDL-Cholesterol level of the subgroup with clear lenses was 1.35mmol/l(SD = 0.35mmol/l) and the mean HDL-Cholesterol level of the subgroup with lens opacities was 1.02mmol/l(SD = 0.27mmol/l). This difference of 0.33mmol/l was highly significant ($p<0.0001$)(Figure 3). In stratifying the subjects according to HDL-C levels, it was clear that above a HDL-C level of 1,5mmol/l the number of subjects with clear lenses increased. This was reversed with levels below 1,5mmol/l (Figure 4). The ODDS RATIO (OR) for this shift to happen was 7.33 (95% CI = 2.06-26.10 p=0.001 for the trend), which predicts that below a HDL-C level of 1,5mmol/l subjects had a seven-fold higher risk of falling in the lens opacity subgroup than those with HDL-C levels above 1,5mmol/l.

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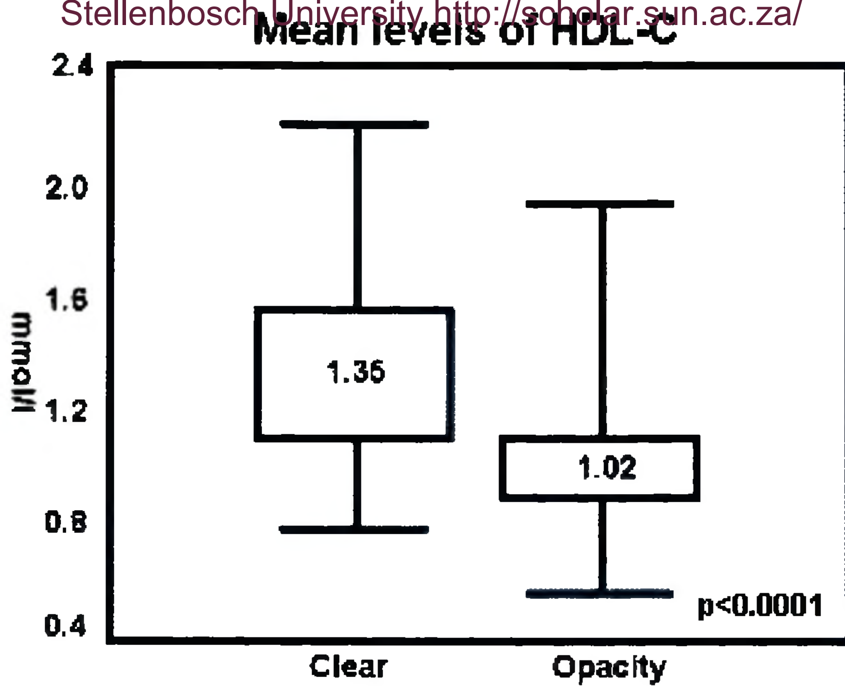

Figure 3. Comparison of the serum HDL-Cholesterol levels (mmol/l) of the two subgroups [(with and without opacities. Difference: ($p<0.0001$)].

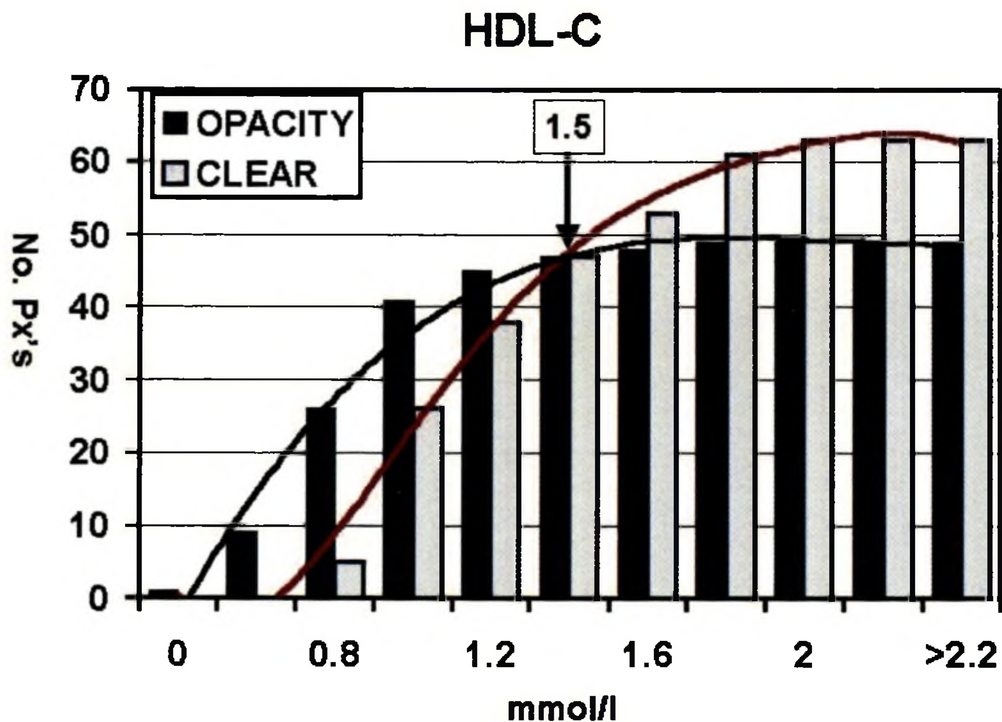

Figure 4. Intra-group stratification of the two subgroups (with and without opacities) according to HDL-Cholesterol levels.

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IV. COMPARISON OF LDL:HDL RATIOS BETWEEN THE TWO SUBGROUPS

Mean LDL:HDL-C ratios were also different between the two subgroups. The mean LDL:HDL-C ratio in the subgroup with clear lenses was 4.67 and in the subgroup with lens opacities 6.24. This difference of 1.57 was highly significant ((p=0.0003)(Figure 5). The OR was 2.35 (95%CI=1.09-5.04, p=0.014 for the trend) which implies that subjects with a LDL:HDL-C ratio below 5 possessed a 2.35 times greater risk of having lenticular opacities than the group with a LDL:HDL-C ratio greater than 5.(Figure 6)

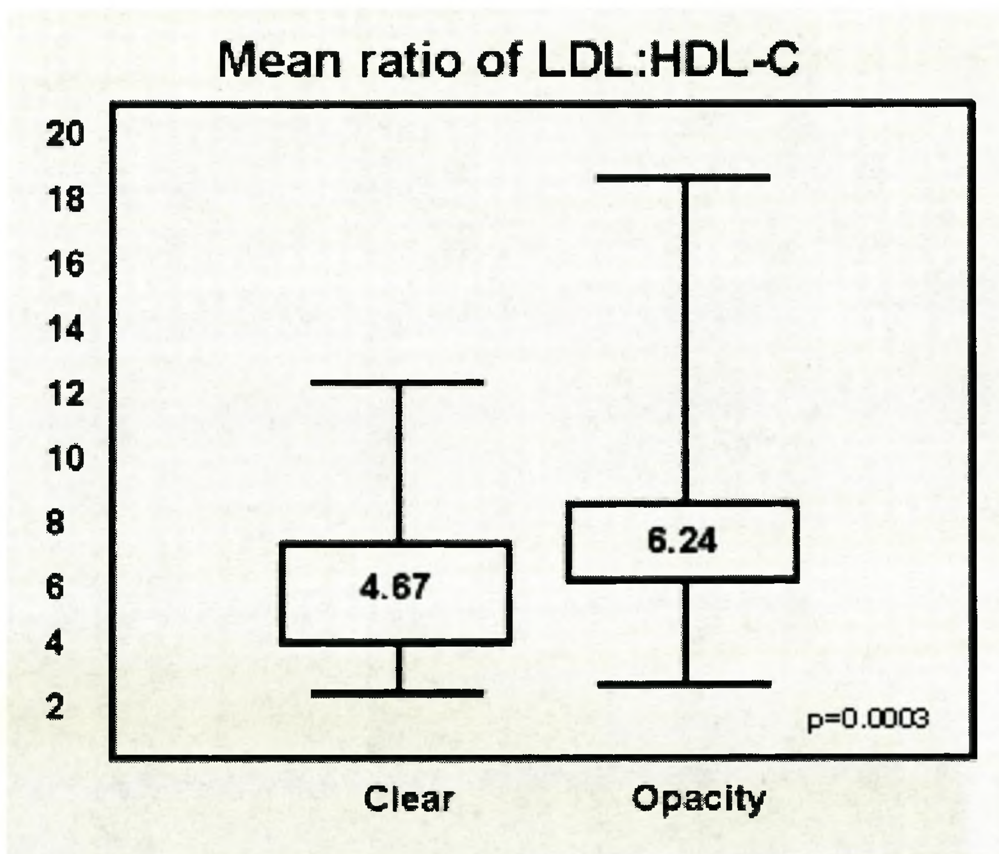

Figure 5. Comparison of the serum LDL:HDL-Cholesterol ratios of the two subgroups [with and without opacities. Difference: (p=0.0003)].

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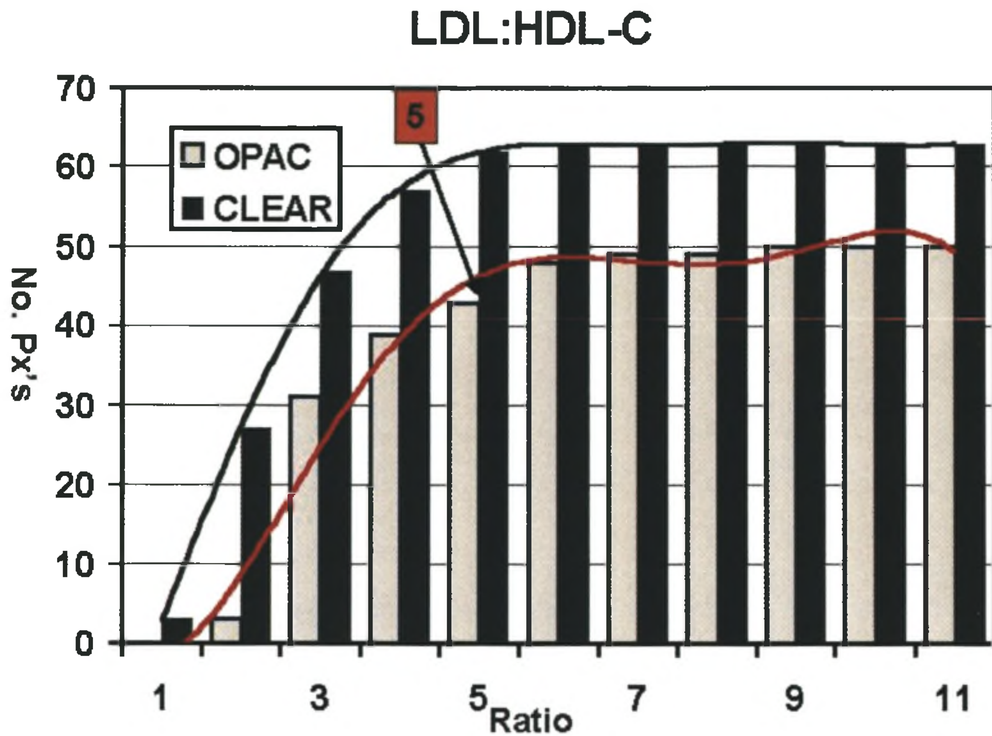

Figure 6. Stratification of the two subgroups according to their LDL:HDL-Cholesterol ratios.

D. DISCUSSION

In many epidemiologic studies, low levels of high density(α-) lipoproteins (HDL) (Hypoalphalipoproteinemia) have been associated with increased coronary artery disease (CAD) risk whereas a high HDL level (> 1.5 mmol/l) is widely considered to be a negative risk factor for the development of CAD[1]. The observations in this study also suggest a clear relationship between low levels (<1.5 mmol/l) of HDL-Cholesterol and the presence of lenticular opacities [OR 7.33 (95% CI=2.06-26.10, p=0.001)].

A LDL:HDL-Cholesterol ratio >5 constitutes another widely excepted risk factor for the development of CAD[2]. Furthermore, the observations in this study group support the relationship between high serum LDL:HDL-Cholesterol ratios (> 5) and lens opacities [OR 2.35 (95%CI=1.09-5.04, p=0.014)].

Therefore, the very same serum lipid components [low HDL (<1.5mmol/l) levels and high LDL:HDL-Cholesterol ratio's (>5)] that have been identified as risk factors for CAD are also in this study for the first time identified as risk factors for lenticular opacification (mainly of the cortical variety).

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The protective effect of HDL-C against CAD lies in the ability of HDL to act as an antioxidant in inhibiting the formation of oxidized LDL[3]. This in turn inhibits the process of atherosclerosis. Could this exact same mechanism be involved in the lens? What evidence exists?

Oxidative damage has been considered as a major factor involved in cataract formation.[4] The lens is chronically exposed to radiation and upon ageing absorbs increasing amounts of ultraviolet light[5]. Increased production of reactive oxygen species is a feature of most, if not all, human disease including cardiovascular disease, cancer and cataract[6]. Dietary antioxidants may be especially important in protecting against human diseases associated with free radical damage to cellular DNA, lipids, and proteins[7].
Hence it has been shown that:

1. Ascorbic acid is an effective water-soluble antioxidant. Epidemiologic evidence suggest that increased ascorbate nutriture is associated with reduced risk of some degenerative diseases, including cataract.
2. High vitamin E intake is associated with decreased risk of coronary heart disease, possibly as a result of inhibition of atherogenic forms of oxidized LDL.
3. Beta-carotene provides protection against lipid peroxidation in humans.

Experimental work demonstrating the cataractogenic effect of oxygen[8] together with the correlation of the incidence of cataracts with solar radiation[9] has led to the notion that intraocular generation of certain active species of oxygen under both photochemical and ambient conditions may initiate a cascade of toxic biochemical reactions, leading ultimately to cataracts and other age-related eye diseases[10,11,12]. Thus, according to this theory, the ambient oxygen itself serves as a pathogen after its derivatization to its more reactive species, commonly referred to as oxygen radicals.

Furthermore, most ocular tissues including the lens, vitreous, and aqueous humours contain detectable amounts of photosensitizers such as riboflavin and kynurenine[13,14]. A small quantity of these photosensitizers can constantly generate superoxide and, hence, other active oxygen species so long as an appropriate activator (electron donor) is available and the reaction solution is exposed to light covering the wavelengths appropriate for photo-activation[15]. These sensitizers can damage and crosslink lens proteins. The concentration of hydrogen peroxide in the aqueous humor is also remarkably high[16] and further increases in cases with cataract as do the levels of H_2O_2 in the lens itself[17]. Extensive oxidation of proteins and accumulation of the oxidised metabolites have also been reported in cataractous lenses. The ability of the eye to deal with these products decreases with age. This is demonstrated by measured decrease of the activity of antioxidant enzymes, particularly superoxide dismutase[18], as well as a decrease in glutathione levels in aged cataractous lenses, accounting for the loss of antioxidant protection[19].

The impact of oxygen free radical toxicity appears far more real in the eye and specifically the lens than anywhere else in the body. The transparency of the cornea, aqueous humor, lens and vitreous allows a unique situation for an incessant photochemical generation of oxygen radicals in all the ocular tissues and the bathing

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fluids, at least during periods of photopic vision. The amount of oxyradicals formed by photocatalysis would be additive to that generated by the normal nonphotochemical reactions.

PATHOGENESIS

'CHOLESTEROL IS BOTH BAD(as oxidant) AND GOOD(as anti-oxidant)'

OXIDATIVE DEGENERATION OF CHOLESTEROL:

Cholesterol can be readily oxidized by a variety of reactive oxygen species yielding several products, some of which possess adverse biological effects[20]. The absolute amount of cholesterol in the lens is not remarkably high, but is concentrated in lens cell membranes. These membranes are known to have the highest cholesterol content of any biological membrane. Cholesterol distribution in the lens appears to follow an unusual pattern concentrating in the pericortical region[21].

As an unsaturated lipid, cholesterol is able to autoxidize. This autoxidation can be initiated by most of the reactive oxygen species. Although the lens contains only small amounts of other unsaturated fatty acids[22], the high concentration of cholesterol in lens membrane may provide the most important substrate for oxidation. The complete or partial structure of over 80 cholesterol oxidation products has already been elucidated[23,24]. Some of these products have been found to possess adverse biological effects including cytotoxicity and mutagenicity[25]. Girao[26] et al (1998) were the first to show that oxysterols (the products of lipid oxydation) accumulate in human cataracts. Significant oxidation of cholesterol may well result in cell injury at least partially compatible with the damage associated with cataract formation.

HDL-CHOLESTEROL AS ANTIOXIDANT:

In other tissues, it has been shown that the activity of membrane ionic pumps is strongly dependent on cholesterol homeostasis[27]. It is conceivable that similar changes in lens membranes could result in electrolytic imbalance disrupting, for example, calcium homeostasis, which could result in cataract formation by a plethora of possible mechanisms.

The assumption that cholesterol oxidation is a stochastic, chemically destructive process may however underestimate the significance of its ubiquitous presence in living cells. It has been suggested in different contexts that cholesterol may act as an antioxidant[28]. Should this be the case in the lens, the accumulation of small amounts of oxysterols in human cataracts could be purposeful (scavenging putative harmful oxidants) and reflect a cholesterol antioxidant role indicating, at the same time, an increased production of oxidants and/or a failure in the antioxidant protective systems in the cataractous lens. Cholesterol would in this case act as a 'sacrificial' antioxidant protecting other membrane components. The high concentrations of cholesterol in the lens would enable it to perform a role in the lens comparable to that ascribed for albumin in the plasma[29]. The lens could in this regard be considered to be 'the Albumin of the eye'.

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Girao et al[30] (1999) were the first to propose that cholesterol may act as an antioxidant. In particular HDL cholesterol, because of it's well known ability to protect the body from oxidative damage found in cardiovascular disease[31]. Girao's study[32], was designed to establish whether HDL-cholesterol acts as an antioxidant in the bovine lens. They found that oxidation of bovine lens membrane results in the production of lipid hydroperoxides, consumption of endogenous vitamin E and formation of cholesterol oxides and concluded that HDL-C present important characteristics generally ascribed to an antioxidant molecule.

The lens cell membrane has the highest concentration of cholesterol in the body. The cholesterol to phospholipid (C/P) mole ratio in the lens ranges between 1 and 4. In contrast, plasma membranes of typical eukaryotic cells have C/P mole ratios between 0,5 and 1,0. The only other known membrane with C/P mole ratios comparable to the lens, are diseased, atherosclerotic, vascular smooth muscle cell membranes. Adequate vision relies on lens transparency, which in turn is severely affected by any change in the lens membrane structure or composition. Altering of the lens-lipid composition or structure may cause lenticular opacities. These lens lipids are prone to oxidative damage. HDL cholesterol acts as an antioxidant in protecting the lens against this oxidative insult. Cholesterol may therefore be regarded both as a *bad* (Oxidant) and as a *good* molecule (Antioxidant).

PHOTOPIC VISION GENERATES FREE RADICALS

The mechanism by which photopic vision, via both a photochemical and non-photochemical pathway, produces free radicals, is summarized in Figure 7. Normal mechanisms exist by which these reactive oxygen species are inactivated, but should these mechanisms fail, cytotoxicity results either directly as damage to cellular DNA, lipids and protein, or indirectly via the oxidation of cholesterol and cholesterol oxidation products. A lack of adequate anti-oxidants, including HDL-Cholesterol, will result in lens damage by the reactive oxygen species produced by daily photopic vision.

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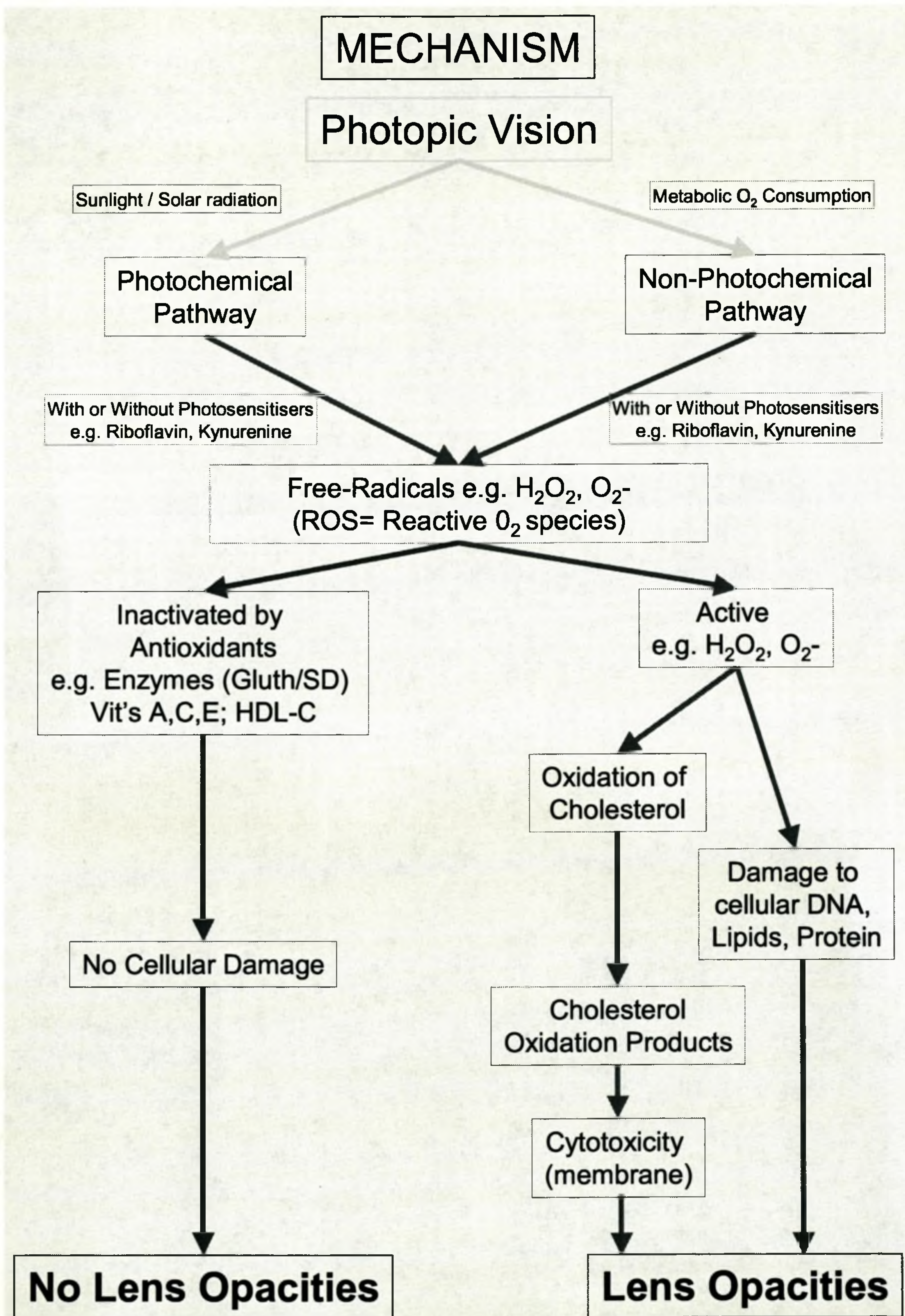

Figure 7. Schematic summary of proposed mechanism by which photopic vision leads to the production of free radicals, cholesterol oxidation and lens opacities.

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CHOLESTEROL AND STEROID CATARACTS

Cholesterol is the parent compound from which all steroid hormones are derived, and provides the basic molecular framework, to which moieties are added, and modifications are made, in the course of the synthesis of functional molecules. It is conceivable that a super-abundance of cholesterol at metabolic sites causes an increase in the rate of hormone synthesis. Since steroids are highly active even in extremely low concentrations, and are known to be cataractogenic, the cataractogenic potential of hypercholesterolemia, identified in the course of the work presented above, may be mediated, at least in part, by a postulated increase in hormone synthesis.

It has recently firmly been established that alpha-crystallin is the principal glucocorticosteroid binding protein in the lens[33]. Furthermore, the epithelial membranes of the bovine lens have been shown to contain a protein capable of binding progesterone with high affinity[34]. Alpha-crystallin is the most plentiful protein in the lens (approximately 50 mg are present in a single human lens) and has a greater tendency than other crystallins to bind covalently to glucocorticosteroids. Consequently, it has been suggested that binding of crystallin to moieties, such as steroids, may cause conformational changes that promote lens opacification and cataractogenesis. Furthermore, it is possible that accumulation of steroids (e.g., dexamethasone), in specific compartments in the tissues of the eye, may elicit an enhanced localised adverse response by promoting binding of steroids to crystallin in that specific locality. Accumulation of steroids in the retrolental spaces of Berger, between the posterior lens capsule, and the anterior hyaloid surface is a case in point. Accumulation of glucocorticosteroids in these spaces may well explain the classic primary appearance of a central posterior subcapsular cataract in patients receiving long-term medication with these agents.

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E. CONCLUSIONS

Total serum cholesterol, triglyceride, and LDL (Low Density Lipoprotein) levels do not statistically correlate with lens opacities in dyslipidemic patients. But low HDL-C levels (<1.5mmol/l) and ah elevated LDL:HDL ratio (>5) present significant cataractogenic risk factors whereas the lens is protected by high HDL-C levels (>1.5mmol/l) and low LDL:HDL ratios (<5) in the dyslipidemic patient. These are exactly the same risk factors that have often been implicated in the development of atherosclerotic circulatory disease.

It is true that low HDL levels can frequently be linked to a genetic predisposition but HDL levels can also be reduced by other factors. These include obesity, sedentary lifestyle, cigarette use, diabetes mellitus, uremia, nephrotic syndrome, and several drugs like thiazide diuretics, retinoids, betablockers, androgenic steroids and most progestational drugs.[35]. Could human age-related cataract therefore be regarded as a preventable condition because of this association of low HDL levels with lifestyle factors? Because these factors are potentially modifiable by lifestyle changes these observations may prove important as the modification of these parameters could constitute an effective mode of prevention or retardation in a subgroup of patients who develop cataracts at an early age.

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CHAPTER 6

CONJUGATE ACETYLATION - A REVIEW

CHAPTER COMPONENTS

A. Introduction
B. Detoxifying Enzyme Systems
C. Biochemistry of Acetylation
D. Arylamine N-transferase in tissues
E. Molecular genetics
F. Population genetics
G. Lifestyle, Environmental Factors and Disease Associations

A. INTRODUCTION

Humans are constantly exposed to foreign chemicals, both man-made and natural. These foreign chemicals, or xenobiotics, may be drugs, industrial chemicals, pesticides, or toxins from plants, animals and molds. Removal of these compounds from the body often relies on enzymatic conversion (i.e. metabolism) in the liver and other tissues to more readily excretable water-soluble forms by biotransformation. Biotransformation can alter the biological effects of drugs and other xenobiotics. For example, some drugs must undergo biotransformation in order to exert their pharmacological effect - in this case, a metabolite is the active drug. On the other hand a xenobiotic can be metabolized to a more toxic compound. In most cases, however, biotransformation terminates the biological effect of a xenobiotic. Thus, biotransforming enzymes are often responsible for both the duration and the intensity of drug action as well as being important in chemical toxicity.

Individuals may differ in their ability to metabolize drugs and other xenobiotics due to inherited variations in the gene sequence of their biotransforming enzymes. The genetic differences, referred to as genetic polymorphisms, can increase or decrease drug metabolism and in some cases, even eliminate a metabolic pathway.

These genetic polymorphisms have been associated with many adverse clinical conditions, but no associations have ever been made between any ocular condition (degenerative or otherwise) and genetic polymorphisms.

The precise cause of cataract is unknown, but it is unlikely that there is a single precipitating event leading to opacification of the lens. It is more likely that many factors influence various aspects of lens metabolism leading to the development of light-scattering centers and loss of transparency.[1]

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B. DETOXIFYING ENZYME SYSTEMS

The liver is the primary site of metabolism (or biotransformation) of foreign chemicals (or xenobiotics). Hepatic function, in turn, is dependent on an adequate and efficient blood supply and adequate metabolic capacity in respect of the specific substrate being metabolised. Hepatic metabolism involves oxidation, reduction or hydrolysis (referred to as Phase I metabolism) or conjugation of an endogenous moiety to the parent xenobiotic or its metabolites (referred to as Phase II metabolism), the latter originating from Phase I metabolism. The endogenous moiety involved in Phase II metabolism may be any one of the following: glucuronate, sulphate, acetate, glycine, or glutathione.

The work presented here is in respect of the conjugative acetylation of isoniazid, used as a marker substrate, wherewith to assess acetylation capacity and acetylator status in a population of patients presenting with age-related lens degeneration.

N-ACETYLATION AS DETOXIFICATION SYSTEM IN HUMANS

N-acetylation is an important route of biotransformation for xenobiotics, which have an aromatic amine (R-NH2), or a hydrazine group (R-NH-NH2). In humans, N-acetylation is mediated by 2 distinct cytosolic enzymes characterized by different physico-chemical properties and substrate specificity: N-acetyltransferase 1 (NAT 1) and N-acetyltransferase 2 (NAT 2). Human NAT 1 and NAT 2 catalyze the acetyl coenzyme A-dependent N- and O-acetylation of primary arylamine and hydrazine xenobiotics and their hydroxylamine metabolites. NAT 2 was for years believed to be characterized by a bimodal distribution so that the population was classified into rapid (or fast) and slow acetylators according to the presence or the absence of an efficient NAT 2 enzyme. The slow acetylator phenotype is the result of mutations in the NAT 2 gene, which decrease NAT 2 activity or enzyme stability. Individuals without these mutations are considered rapid acetylators and have the 'wild-type' or high activity alleles[2].

It has recently been firmly established that the polymorphism displayed by NAT 2 is in fact trimodal in humans for substrates like sulfamethazine[3] and isoniazid[4]. Human populations may therefore be divided into rapid, intermediate, and slow acetylator phenotypes. This has significant implications in clinical disease associations, drug therapy and development, and toxicology.

The acetylation polymorphism (NAT 2) was discovered over 45 years ago following differences observed in tuberculosis patients to isoniazid (Isonicotinic acid hydrazide) toxicity.[5] As such it holds a special place as one of the first described examples of a pharmacogenetic defect affecting xenobiotic biotransformation capacity in human populations. Subsequently, the differences in isoniazid toxicity were attributed to genetic variability in N-acetyltransferase, a cytosolic phase II conjugation enzyme primarily responsible for deactivation of isoniazid.[6] The polymorphism was termed the "isoniazid acetylation polymorphism" for many years until the importance of the polymorphism in the metabolism and disposition of other drugs, chemical carcinogens, mutagens and cytotoxic substances was fully appreciated.[7]

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Aromatic amines and hydrazines (N-acetylation) and N-hydroxy-aromatic and heterocyclic amines (O-acetylation) are both examples of acceptor substrates that, in general, are deactivated (N-acetylation) or activated (O-acetylation) by NAT 1 and/or NAT 2 .[8]

DRUG AND XENOBIOTIC MEDIATED TOXICITY MECHANISMS.

(Why SLOW can be 'good' or 'bad' and RAPID can be 'bad' or 'good'.)

With regard to the susceptibility of the phenotypes to drug or xenobiotic toxicity, the following scenarios can be considered:[9]

The toxicity of the drug is caused by the parent compound and the elimination of the drug proceeds exclusively via the polymorphic enzyme. No alternate pathways of biotransformation are available. Thus the slow acetylator/poor metabolizer phenotype will be more prone to such a type of toxicity since, at the same level of exposure, this phenotype will accumulate the drug as a result of impaired metabolism (e.g. INH polyneuropathy, perhexiline polyneuropathy, pesticide induced Parkinson's disease).

The polymorphic pathway is the major route of detoxification but impairment of this pathway shifts the metabolism to an alternate pathway via which a reactive intermediate is formed. In such a situation the slow acetylator/poor metabolizer phenotype constitutes a major risk factor for toxicity (e.g. INH hepatotoxicity).

The toxicity is mediated by a reactive intermediate generated by a polymorphic enzyme. Hence rapid acetylators and extensive metabolizers are at a much higher risk than slow acetylators/poor metabolizers to develop toxicity or cancer (e.g. Bronchial carcinoma in smokers)

TOXICOLOGY

From a toxicological perspective, both NAT 1 and NAT 2 are able to catalyze acetylation reactions that can be considered protective as well as those that contribute to increased chemical reactivity and toxicity. Acetylation of the nitrogen atoms of primary aromatic amine or hydrazine functional groups produces arylacetamides or hydrazides, respectively, which are chemically stable. On the other hand when NAT 1 or NAT 2 acetylates the oxygen atoms of certain hydroxylamines (which can arise from amines by cytochrome P450-dependant oxidation), an acetoxy ester is produced that may be chemically unstable. Cleavage of the N-O bond thus yields an electrophilic nitrenium ion that can bind to nucleophilic sites on intracellular macromolecules, such as DNA bases or proteins. This binding can ultimately result in genotoxicity or cytotoxicity. Therefore, the relative kinetic selectivities of NAT 1 and NAT 2 for N- vs O-acetylation of particular homo- and heterocyclic arylamines and their oxidized metabolites, will also be important predictors of the toxicity of such compounds.[10]

Any effect upon lens protein therefore is most likely strongly dependent on exposure to arylamine and hydrazine substances. Our inability to determine exposure levels with sufficient accuracy may be the most important limiting factor in epidemiological lens studies.

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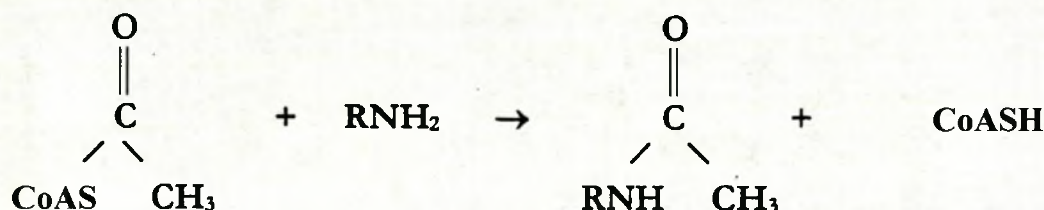
C. BIOCHEMISTRY OF ACETYLATION

THE ACETYLATION REACTION

When a drug or substance becomes acetylated it refers to a reaction where an acetyl radical (-CH_3CO) of acetyl Co-enzyme A is transferred to form an amide bond with the nitrogen atom of primary amines, amino acids, hydrazines (R-NH-NH_2) or sulfonamides.



CoA = Co-enzyme A; R = organic molecule, generally a heterocyclic amine, S=Sulphur Acetyl-CoA (active acetate) is the acetyl donor. CoA = Co-enzyme A; R = organic molecule, generally a heterocyclic amine, S =

The above reaction takes place in the human and in some animals, where the arylamine acetyltransferases (NAT 1 and NAT 2) present in the cytosol of various tissues, e.g. Kupffer cells of the liver, reticulo-endothelial cells of the lung, spleen and mucosa of the gastro intestinal tract[11], catalyse the transfer of an acetyl-group from acetyl CoA to a primary amino group on the substrate (Phase II conjugation). Examples of acetylation reactions include that of isoniazid and the sulphonamides.

D. ARYLAMINE N-TRANSFERASE IN OCULAR and OTHER TISSUES

Very little research has been done on the presence of the N-Acetyltransferase (NAT) system in the vertebrate eye. The presence of N-Acetyltransferase activity in the human eye has not yet been documented. More is known about it in a few animal models.

LENS

NAT activity was identified and partially characterized in the bovine lens. According to size-exclusion HPLC, the molecular mass of the arylamine NAT is approximately 30-kDa. This arylamine NAT in the bovine lens acetylates para-aminobenzoic acid thereby demonstrating a monomorphic pattern of N-acetylation. It also demonstrates low sensitivity to methotrexate inhibition. This could therefore be a NAT -1 enzyme. Gaudet et al concludes that NAT could be involved in lenticular detoxification of both endogenous amines and exogenous drugs.[12]

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RETINA

NAT activity was demonstrated in the frog retina (Rana perezi) by Alonso-Gomez et al[13]. They found that arylamine N-acetyltransferase (A-NAT) activity is distributed in both neural retina and choroid-pigment epithelium (RPE) complex but showing a 10-fold higher specific activity in neural retina. In contrast arylalkylamine N-acetyltransferase (AA-NAT) activity is restricted to neural retina.

Gaudet et al identified and characterized A-NAT activity in bovine retina[14] and RPE cells.[15] This A-NAT demonstrated a monomorphic pattern of acetylation since it acetylates rho-amonobenzoic acid rather than sulfamethazine. It also demonstrates a low sensitivity to methotrexate inhibition. This could therefore also be a NAT -1 enzyme.

ANTERIOR SEGMENT TISSUE

Campbell et al[16] studied the activity of arylamine acetyltransferase with p-aminobenzoic acid (PABA), sulfamethazine (SMZ), and aminoxolamide as substrates in rabbit tissue homogenates of the corneal epithelium, -stroma, -endothelium and iris-ciliary processes. They concluded that there is a significant amount of acetyltransferase activity in the mentioned ocular tissues of the rabbit with these three substrates, indicating that acetylation may also be occurring for other arylamine drugs in the eye.

OTHER TISSUES

Functional enzyme activity measurements have generally led to the conclusion that NAT 2 is expressed selectively in liver and gut, whereas NAT 1 activity can be detected at high levels in almost all human tissues[17]. In situ hybridization and immunohistochemical studies have suggested that both NAT 1 and NAT 2 are broadly distributed among various human tissues[18]. Acetylation of platelet membrane proteins either in vitro or in vivo reduces membrane fluidity[19], and acetylation of circulatory proteins such as hemoglobin and albumin inhibits glycation of these molecules[20].

E. MOLECULAR GENETICS OF NAT 1 AND NAT 2

N-ACETYLTRANSFERASE GENE NOMENCLATURE

The critical need to standardize and widely disseminate consensus gene nomenclature has been recently reviewed by Nebert[21]. A consensus NAT nomenclature was published in 1995[22] and the First International Workshop on the Arylamine N-Acetyltransferases (A N-NAT) was held in Cairns, Australia in October 1998. A committee has been elected to maintain a website providing an up-to-date listing of arylamine N-

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acetyltransferase alleles[23]. A clear consensus has been reached that the official symbol for the gene should be *NAT* (case-insensitive).

NAT 1 and *NAT* 2 are products of single, intronless proteincoding exons of 870-bp (base pairs) open reading frames encoding 290 amino acids.[24,25] *NAT* 1 and *NAT* 2 and a pseudogene, *NAT* P are located on human chromosome 8p22[26]. *NAT* 1 and *NAT* 2 genes are separated by at least 25 kb.[27] A third *NAT* gene (*NAT* 3) has been identified in the mouse[28]. *NAT* 1 and *NAT* 2 share 87% nucleotide homology in the coding region, yielding 55 amino acid differences. Whereas *NAT* 1 derives its entire transcript from a single exon, *NAT* 2 mRNA is derived from both the protein-coding exon and a second noncoding exon of 100 bp located about 8kb upstream of the translation start site.[29,30].

Seven missense (G191A, T341C, A434C, G590A, A803G, A845C, and G857A) and four silent (T111C, C282T, C481T, and C759T) substitutions have been identified thus far in the *NAT* 2 coding exon i.e. only 11 sites within the *NAT* 2 coding region where nucleotide substitutions have been observed. Five of these are capable of producing the slow acetylator phenotype by impairing *NAT* 2 function. *NAT* 2 alleles containing the G191A, T341C, A434C, G590A and/or G857A missense substitutions are most commonly associated with the slow acetylator phenotype. The four most common of these are *NAT* 2 *5(341), *NAT* 2 *6(590), *NAT* 2 *7(857) and *NAT* 2 *14(191)[31]. *NAT* 2 *4 is considered the wild-type high activity (rapid acetylator) allele because of the absence of any of these substitutions. However *NAT* 2 *4 is not the most common allele in many ethnic groups, including Caucasians and Africans. Striking ethnic differences in the frequencies of these missense substitutions are responsible for the corresponding ethnic differences in frequency of slow acetylator alleles. An example is the G191A substitution common to the *NAT* 2 *14 gene cluster, which is present in African Americans and native Africans, but is virtually, absent in Caucasian populations.

To date 26 different *NAT* 2 alleles have been identified in human populations. Genetic polymorphism is defined as allele frequency reflecting a nucleotide diversity greater than 0.01. Some of the nucleotide substitutions and corresponding *NAT* 1 and *NAT* 2 alleles are rare and reflect nucleotide diversity with a frequency of only 0.003-0.005. Moreover some of the nucleotide substitutions are silent (do not change primary amino acid sequence) and may therefore be considered insignificant.[32]

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F. POPULATION GENETICS

Ethnic differences in acetylator profiles have been well reported in the literature. What follows is a local chelation of the current literature on the global distribution of acetylator status in both a bimodal and trimodal fashion.

AVERAGE BIMODAL DISTRIBUTION OF ACETYLATOR STATUS (%)

United States of America	65,0 (Slow)	35,0 (Fast)
Europe	48,4 (Slow)	51,6 (Fast)
East Asia	12,0 (Slow)	88,0 (Fast)
Middle East	63,6 (Slow)	36.4 (Fast)
Africa	11,0 (Slow)	89,0 (Fast)

The bimodal distribution in the USA is on par with the Middle East countries. The Caucasians in Europe have an almost equal distribution between slow and rapid acetylators. East Asia and Africa compare favorably with almost 90% of the population manifesting as rapid acetylators.

AVERAGE TRIMODAL DISTRIBUTION OF ACELATOR STATUS (%)

United States of America	40,0 (Slow)	40,0 (Intermediate)	20,0(Fast)
England	55,0 (Slow)	35,0 (Intermediate)	10,0(Fast)

The highest trimodal slow acetylator distribution is found in England and the lowest of this group in the United States. The opposite finding counts for the fast trimodal population, while the United States have the highest percentage intermediate acetylators compared to the group found in England.

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G. LIFESTYLE, ENVIRONMENTAL FACTORS AND DISEASE ASSOCIATIONS WITH N-ACETYLTRANSFERASE (NAT 2)

Several lifestyle and environmental factors have been suggested to impact on the acetylation status of individuals. The best studied is the association between well-done meat and colon cancer on the one hand and smoking and bladder carcinoma on the other. Several diseases/conditions have also extensively been studied and attempts made to link them to the acetylation status of individuals. The work on these conditions are summarized below (Table 1) and grouped into associations with rapid acetylators, associations with slow acetylators, conditions where no association could be found and diseases where controversy still exists.

LIFESTYLE AND ENVIRONMENTAL FACTORS

DIET and **COLORECTAL** carcinoma: NAT 2 is known to catalyze the formation of mutagenic products from foodstuffs, especially cooked meat and fish. Roberts-Thomson et al[33] studied the adenoma and cancer risk in relation to meat intake and acetylator status in a case-control study. They found that the rapid acetylator phenotype was associated with a 2.5 and 8.9 higher risk for adenoma and colorectal carcinoma respectively in their group of patients <64 years. The risk of adenoma or cancer increased with increasing intake of meat in rapid but not in slow acetylators. The suggested mechanism is that carcinogenic heterocyclic amines found in well-done cooked meat products are activated by NAT enzymes to genotoxic compounds that can form DNA adducts in the colon epithelium. Chen [34]studied participants in the prospective Physicians' Health Study - 212 men who were subsequently diagnosed with colorectal cancer during 13 years of follow-up along with 221 controls. Among those men who were rapid acetylators, consumption of >1 serving of red meat per day was associated with a relative risk of 5.82(95% CI, 1.11-30.6) compared with consumption of <or = 0.5 serving per day (P, trend = 0.02). These results suggest that polymorphisms in the NAT genes confer differential susceptibility to the effect of red meat consumption on colorectal cancer risk

SMOKING and **BLADDER CANCER**: The first association between slow acetylator phenotype and urinary bladder cancer was reported 20 years ago[35]. Associations between slow acetylator phenotype(s) and urinary bladder cancer are strongest in studies in which there are documented exposures to aromatic amine carcinogens. Aromatic amines from cigarette smoke is a recognized risk factor for bladder cancer. 4-Aminobiphenyl (ABP) is a potent bladder carcinogen that is N-acetylated as an overall detoxification step. In Birmingham a significant excess of genotypic slow acetylators was found in those groups of bladder cancer patients exposed to arylamines as a result of their occupation or who were cigarette smokers.[36]

SMOKING and **HEAD AND NECK** cancer: Gonzales[37] of Spain analyzed the allele frequencies for NAT 2 in patients with head and neck squamous cell carcinoma, which is strongly associated with tobacco consumption.

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They concluded that slow NAT 2 activity is a risk factor possibly leading to the development of head and neck cancer in response to tobacco carcinogens.

ALCOHOL: Rodrigo of Spain[38] conducted a case-control study in which 120 alcoholic cirrhotics, 30 long-term non-cirrhotic alcoholics, and 200 healthy controls were genotyped for NAT 2 polymorphism. They found that individuals homozygous for the NAT 2 *5 allele [the most frequent slow acetylator (SA) allele], were at a significantly decreased frequency among the cirrhotic patients compared with controls (9% versus 16%; P=0.042). The frequency of this SA genotype was significantly increased (40%) in long -term alcoholics who did not develop cirrhosis (P=0.0041 compared with controls; P=0.000017). They therefore concluded that NAT 2 activity may be a factor that determines the risk of developing alcoholic liver cirrhosis, and slow acetylators would be protected.

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DISEASES/CONDITIONS ASSOCIATED WITH RAPID ACETYLATORS

Table 1. Disease/Conditions associated with the different tempo's of acetylation.

Rapid	Slow	No Association	Controversial
1) Colorectal cancer[39,40,41,42,43,44]	Familial Parkinson's disease[45,46]	Alzheimer's disease[47,48].	Breast carcinoma [49,50,51,52,53,54,55,56,57]
2) Type I Diabetes mellitus[58,59,60]	Epilepsy[61]	Idiopathic Parkinson's disease[62,63,64,65]	Head & Neck cancer[66,67,68,69]
3) Lung cancer[70,71,72,73]	Gilbert's disease[74]	Type II Diabetic nephropathy[75]	Graves disease[76]
4) Breast disease[77]	Endometriosis[78]	Prostate carcinoma[79,80]	
5) Glomerulonephritis[81].	Bladder carcioma[82,83,84]	Hematological diseases[85,86]	
6) Laryngeal carcinoma[87]	Renal carcinoma[88]	Pancreatic disease[89]	
7) Phenylketonuria[90]	Malignant mesothelioma[91]		
8)	Esophageal carcinoma[92]		
9)	Asthma[93]		
10)	Allergic/atopic diseases[94,95,,96]		
11)	Autoimmune diseases[97,]		

The pivotal question that will hence be addressed in the next chapter will be whether Acetylator geno- and phenotype can be a genetic marker for age related cataract?

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CHAPTER 7

AGE RELATED CATARACTS AND ACETYLATION STATUS – A POPULATION STUDY

ABSTRACT

PURPOSE

To study the relationship between age related cataracts in humans and the acetylation status of the affected person in a well defined population of the Western Cape Province of South Africa.

METHODS

One hundred and thirty nine(139) adult patients of both genders with age related cataract presenting for lens extraction and intra-ocular lens implantation surgery and one hundred and fourteen(114) adult controls were pheno-and genotyped to determine their acetylation status as accurately as possible. Absolute concordance between phenotype [determined by the High-performance Liquid Chromatographic (HPLC) method using Isoniazid (INH) as a test substrate], and genotype [determined by the Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) technique], was required for inclusion into the study.

RESULTS

The distribution of polymorphic acetylator phenotypes and genotypes in the two groups determined in a trimodal fashion were as follows:

STUDYGROUP (n=139)	**CONTROLS (n=114)**
Fast=22(16%)	Fast=26(22,8%)
Inermediate=55(40%)	Intermediate=54(47,4%)
Slow=62(45%)	Slow=34(29,8%)

Whole group comparison using the chi-squared test confirmed a difference between the two groups. This was significant when comparing the phenotypes (p=0.047) as well as the allele frequencies in accordance with Hardy-Weinberg principles (p=0.047). Genotype distributions were in Hardy-Weinberg equilibrium.

Comparing the frequencies of the acetylator subtypes of the studygroup and the controls, the cases contained a greater proportion of slow acetylators than the controls (p=0.008). The differences in the fast and intermediate groups was not significant (p=0.08 and p=0.11 respectively).

Allele frequencies were determined by counting alleles. The cases contained 99 fast and 179 slow alleles (total = 278) and the control group consisted of 106 fast and 122 slow alleles (total = 228). When expressed as

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percentages, a significant decrease in the fast alleles and an equally significant rise in the slow alleles in the cataract group was evident (p=0.0066).

Odds ratios were determined to assess the risk ratio of cases in the cataract group compared to the control group for the slow acetylator state. This was determined both for the phenotypes (OR 2.16, CI 0.98-4.77, p trend=0.028) and the genotypes (OR 2.44, CI 1.05-5.66, p trend=0.019).

CONCLUSION

The search for etiological mechanisms of cataract in humans continues. Genetic mechanisms that may contribute to modification and condensation of lens protein should be considered. This study suggests that the slow acetylator pheno- and genotype may be regarded as a risk indicator for age related cataract in the studied population.

A. INTRODUCTION

This study will examine ***conjugate acetylation*** and human cataracts as manifested in a well defined population. It will attempt to show that conjugate acetylation as a detoxifying metabolic process is strongly suspected as being involved in human cataractogenesis. Data is presented that significantly links the slow acetylator pheno- and genotype to adult onset age-related cataract.

Humans are constantly exposed to foreign chemicals, both man-made and natural. These foreign chemicals, or xenobiotics, may be drugs, industrial chemicals, pesticides, or toxins from plants, animals and moulds. Removal of these compounds from the body often relies on enzymatic conversion (i.e. metabolism) in the liver and other tissues to more readily excitable water-soluble forms.

Individuals may differ in their ability to metabolize drugs and other xenobiotics due to inherited variations in the gene sequence of their biotransforming enzymes. These genetic differences called *genetic polymorphisms*, can increase or decrease drug metabolism and in some cases, even eliminate a metabolic pathway. These genetic polymorphisms have been associated with a wide array of clinical conditions. **Slow** acetylator status has been associated with conditions such as urinary bladder cancer[1,2,3,4,5,6], epilepsy[7], Gilbert's Syndrome[8], endometriosis[9], renal cell carcinoma[10], malignant mesothelioma[11,12], esophageal carcinoma[13], asthma[14], allergic and atopic disease[15] and autoimmune diseases such as systemic lupus erythematosis (SLE) and systemic sclerosis (SSc)[16]. **Rapid** acetylator status on the other hand has been associated with conditions such as colorectal cancer [17,18,19], type 1 diabetes mellitus[20], lung cancer[21], glomerulonefritis[22], benign breast disease[23], laryngeal cancer[24] and phenylketonuria[25]. However, no clinical association has to our knowledge been made between degenerative ocular conditions such as cataract and age-related macular degeneration on the one hand and genetic polymorphisms on the other.

Cataracts in humans occurs worldwide, but especially in developing countries. It is one of the major causes of visual impairment eventually leading to blindness if left unoperated. It is now well established that the etiology of human cataract formation is multifactorial. This is apparent by the association of cataracts with a number of clinical conditions. Cataract can also be induced by nutritional deficiency of certain amino acids such as

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tryptophan, proteins (hypoproteinemia) and hypovitaminosis-B_2.[26] Exposure to environmental factors such as various wavelengths of ionizing as well as nonionizing radiations, heavy metals, sunlight, cigarette smoke, oxygen and oxyradicals have all been associated with cataract formation.[27] It is most likely that cataract result from interaction between genetic and environmental factors and hence the fact that age probably is recognized as the most significant associated factor amongst patients in the above 50 year old group.

Because N-acetylation is involved in a wide variety of detoxification processes of inter alia environmental chemicals, this study was conducted to examine the relationship between the acetylator pheno- and genotype in a group of patients with mature age-related cataract in a well-defined segment of the population of the Western Cape region of South Africa. The coloured (mixed race) population is the largest of the ethnically distinct subgroups that inhabit the region. The South African population of mixed ancestry includes Malay, Khoisan, Negriod and Caucasoid stock. Distribution of polymorphic *N*-Acetyltransferase genotypes and phenotypes in this population has previously been well documented by us[28]. Hence this well studied and special population group was selected for the purpose of this study.

B. MATERIALS AND METHODS

SUBJECTS

One hundred and thirty nine (139) adult patients of both genders aged 50 and above, with classic mature age-related cataract presenting for lens extraction and intra-ocular lens implantation surgery were enrolled in the study for which approval was obtained from the Ethics and Research Committee of the University of Stellenbosch. Patients were included in the trial if they perceived themselves to be coloured (of mixed race) and if this was verified by at least one independent observer. The South African population of mixed ancestry (including Malay, Khoisan, Negroid and Caucasoid stock) is referred to as "coloured" throughout this study[29]. Care was taken to exclude all patients with well-recognized etiological factors for cataract formation such as diabetes mellitus, previous ocular trauma, metabolic and/or inherited diseases. The patient sample therefore consisted of cases with age-related cataracts of unknown etiology.

DOSAGE AND SAMPLING

Patients presenting for routine cataract surgery were admitted to the ophthalmology ward, clinically evaluated and consent was obtained on the day prior to the scheduled surgery. On the day of surgery a dosage of 10 mg/kg isoniazid (INH), accurately measured for each patient individually, was administered orally. The oral dose of medication pre-operatively was only possible because all cases were operated on under local anesthesia and hence were not restricted to being nil per mouth. Venous blood samples were subsequently taken at two, three, four and five hours post-dosage. These samples, five milliliters in all instances, were collected in EDTA tubes, chilled and delivered to the laboratory on ice and then used for determining both the acetylator pheno- and genotype of each patient.

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The first 139 consecutive cases with absolute concordance between the phenotype (as determined by INH metabolism) and genotype {determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)} were analyzed. The same requirement of absolute concordance applied to the control group.

PHENOTYPING METHODS

Phenotyping was effected by means of the High-performance Liquid Chromatographic (HPLC) method described by Seifart *et al*[30] in 1995, using INH as a test substrate.

GENOTYPING METHODS

(Refer to Appendix A for detailed methodology.)

In this study peripheral whole blood (5 ml) was collected in EDTA tubes and stored at -20°C until required for the DNA analysis. Genomic DNA was then extracted from each whole blood aliquot, and used in the Polymerase Chain Reaction (PCR) for the specific amplification of the gene sequence of the arylamine *N*-acetyltransferase(*NAT2)* enzyme. The 1000 base pair (bp) PCR product was subsequently analysed using the Restriction Fragment Length Polymorphism (RFLP) technique. Ten microliter aliquots of the 1000 bp PCR product was restricted with the four restriction enzymes *Bam*HI, *Kpn*I *Msp*I and *Taq*I in order to identify the specific/characteristic allelic variations of *NAT2*.

The restriction fragment profiles of each enzyme were resolved by gel electrophoresis using Metaphor agarose, after which the gels were stained with Ethidium Bromide to delineate the specific *NAT2* allelic variants. These enzymes cause a corresponding phenotypic change in the *NAT2* enzyme activity, and enabled us to obtain a good genotype-phenotype correlation of greater than 90%. The results were scored according to the nomenclature of Vatsis et al.[31] In addition to these four enzymes, the *NAT2* PCR product was also restricted with a further two enzymes (DdeI and FokI) which further improved the genotype-phenotype correlation in the patient cohort. These latter enzymes delineate silent mutations in NAT2 which do not result in a change in protein activity. Figure 1 summarizes the genotyping method.

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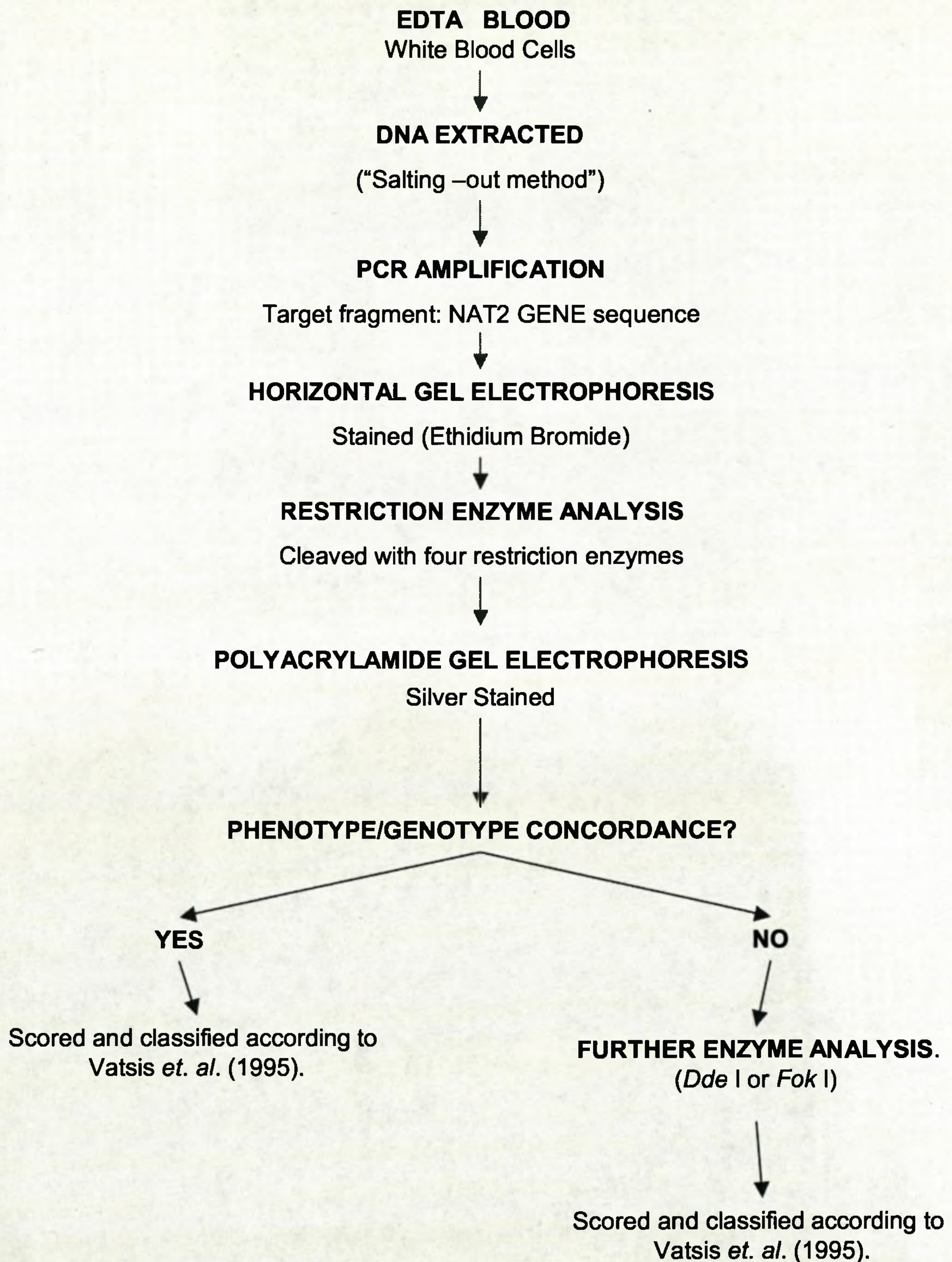

Figure 1.

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STATISTICAL ANALYSIS

All stastics were generated using the Statistica™ 1984-2000 (Release 5.5) by StatSoft, Inc, USA.

C. RESULTS

DATA PROCESSING

The data presented in this section has been processed to support the following:

I. The significant differences that exist between the two study groups (cases and controls) as whole units when stratified according to fast, intermediate and slow acetylator pheno- and genotypes. (Tables 1-6)

II. The clear trimodal INH eliminator pattern of distribution that exists in the cataract group. (Figure 2 & 3)

III. The frequency change towards the slow acetylator state in the cataract group using both the phenotype and alleel distribution frequencies. (Figure 4 – 6)

IV. The *odds ratio* which determines that a relationship exists between age related cataracts and the slow acetylator state and to describe the *strength* of this relationship amongst the different subgroups. (Tables 7-9 and Figures 7& 8)

WHOLE GROUP DATA

The study population consisted of one hundred and thirty nine (139) consecutive cases (50 years and older) with mature age-related cataract with absolute concordance in a trimodal pattern between the INH eliminator phenotype and the *NAT 2* genotype, compared to one hundred and fourteen (114) controls meeting the same criteria.

The cataract group represented the typical age-related cataract population as far as age (mean = 67.4 years and median 66.4 years where the youngest patient was 50.16 and the oldest 86.58 years respectively), mass (mean = 63.15kg and median = 62.5kg) and gender {males 56 (40%) and females 83 (60%)} distribution is concerned.

The control group included 114 adult (older than 18 and younger than 60 years), mixed race patients of both genders who perceived themselves to be coloured and concented to the donation of blood samples.

The distribution of polymorphic acetylator genotypes and phenotypes in the two groups is shown in Table1 (cases) and Table 2 (controls). Genotypically the subjects were classified as **fast** acetylators if their genotype contained WT/WT (two wild type) alleles, **intermediate** if their genotype contained the WT/M (one wild type and mutant) alleles and **slow** if they contained the M/M (two mutant) alleles. The classification was effected by means of the internationally accepted Vatsis Classification.(Ref 31)

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Table 1. Genotype and Phenotype distribution in Cataract Cases: N=139

Group Classification	Vatsis	Genotype	Allele combinations	Phenotypic group totals
Fast group	NAT2*4/*4	F1 F1	22 (16%)	22 (16%)
Intermediate group	NAT2*4/*5	F1 S1	36 (26%)	
	NAT2*4/*6A	F1 S2	16 (12%)	
	NAT2*4/*7	F1 S3	3 (2%)	
		F1 S4	0	55 (40%)
Slow group	NAT2*5	S1 S1	19 (14%)	
	NAT2*5/*6A	S1 S2	21 (15%)	
	NAT2*5/*7	S1 S3	4 (3%)	
	NAT2*5/*14A	S1 S4	2 (1%)	
	NAT2*6A/*6A	S2 S2	9 (6%)	
	NAT2*6A/*7	S2 S3	3 (2%)	
	NAT2*6A/*14A	S2 S4	2 (2%)	
	NAT2*7/*7	S3 S3	1 (1%)	
	NAT2*7/*14A	S3 S4	1 (1%)	
	NAT2*14A/*14A	S4 S4	0	62 (45%)

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Table 2. Genotype and Phenotype distribution in Controls:N=114

Group Classification	Vatsis	Genotype	Allele combinations	Phenotypic group totals
Fast group	NAT2*4/*4	F1 F1	26 (22,8%)	26 (22,8%)
Intermediate group	NAT2*4/*5	F1 S1	20 (17,5%)	
	NAT2*4/*6A	F1 S2	25 (21,9%)	
	NAT2*4/*7	F1 S3	3 (2,6%)	
		F1 S4	6 (5,3%)	54 (47,4%)
Slow group	NAT2*5	S1 S1	9 (7,9%)	
	NAT2*5/*6A	S1 S2	10 (8,8%)	
	NAT2*5/*7	S1 S3	4 (3,5%)	
	NAT2*5/*14A	S1 S4	1 (0,9%)	
	NAT2*6A/*6A	S2 S2	3 (2,6%)	
	NAT2*6A/*7	S2 S3	3 (2,6%)	
	NAT2*6A/*14A	S2 S4	2 (1,8%)	
	NAT2*7/*7	S3 S3	0	
	NAT2*7/*14A	S3 S4	1 (0,9%)	
	NAT2*14A/*14A	S4 S4	1 (0,9%)	34 (29.8%)

I. WHOLE GROUP COMPARISON

The chi-squared test ($\chi^2 = \Sigma$ (O-e)^2/e) was used to determine whether differences exist between the two groups as whole units when stratified according to fast, intermediate and slow pheno- and genotypes. (Table 3). The squared difference (df) between the two groups was 6.0984. Using a 5% significance level, the rejection region is $\chi^2 > \chi^2\alpha$, (r-1)(c-1) = $\chi^2$0.05, 2 =5.99. Because χ^2 = 6.0984 the null hypothesis was rejected in favor of a difference between the cases and controls. (p=0.047).

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Table 3. Observed and expected values of cases and controls.

Observed value	**F**	**I**	**S**	**Total**
Controls	26	54	34	**114**
Case	22	55	62	**139**
Total	**48**	**109**	**96**	**253**
Expected value	**F**	**I**	**S**	**Total**
Controls	21.63	49.11	43.26	**114**
Case	26.37	59.89	52.74	**139**
Total	**48**	**109**	**96**	**253**

ALLELE FREQUENCY COMPARISON (HARDY-WEINBERG)

The allele frequencies were determined by counting alleles and genotype frequencies by calculating sample proportions. This was done in accordance with Hardy-Weinberg principles and the Hardy-Weinberg formula {p^2[SS]:2q(1q)[FS]:$(1q)^2$[FF]}. The two groups were then compared. Table 4 (cases) and Table 5 (controls). Genotype distributions were in Hardy-Weinberg equilibrium.

Table 4. Allele frequencies - Cases:

Acetylator		**Contributions Genotypes to Frequencies**	**Of allele**	**Genotype**	**Frequencies**
Phenotype	**Genotype**	**S*(q)**	**F1(1-q)**	**Observed**	**Calculated**
Fast	F1 F1	-	0.158	0.158	0.127
Intermediate	F1 S1	0.198	0.198	0.396	0.458
Slow	S1 S1	0.446	-	0.446	0.415
Totals	1.000	0.644	0.356	1.000	1.000

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Table 5. Allele frequencies - Controls:

Acetylator		Contributions Genotypes to Frequencies	Of allele	Genotype	Frequencies
Phenotype	**Genotype**	**S*(q)**	**F1(1-q)**	**Observed**	**Calculated**
Fast	F1 F1	-	0.228	0.228	0.216
Intermediate	F1 S1	0.237	0.237	0.474	0.498
Slow	S1 S1	0.298	-	0.298	0.286
Totals	1.000	0.535	0.4645	1.000	1.000

The chi-squared test ($\chi^2 = \Sigma\ (O-e)^2/e$) was again used to determine whether differences in allele frequencies exit between the two groups. This test resulted in $\chi^2 =$ **6.0978** (Table 6). Using a 5% significant level the rejection level was 5.99. But because $\chi^2 = 6.0978$, we could once again conclude that a significant difference existed between cases and controls (p=0.047).

Table 6. Allele frequencies Hardy-Weinberg Model.

Observed value	F	I	S	Total
Controls	24.6	56.7	32.6	**114**
Case	17.7	63.7	57.7	**139**
Total	**42**	**120**	**90**	**253**
Expected value	**F**	**I**	**S**	**Total**
Controls	19.1	54.2	40.7	**114**
Case	23.2	66.1	49.6	**139**
Total	**42**	**120**	**90**	**253**

II. TRIMODAL INH ELIMINATOR PATTERN

Using the first order elimination constant of INH as discriminant, we could clearly demonstrate the trimodal pattern of distribution of acetylator phenotypes applicable to the cataract group. (Figure 2).

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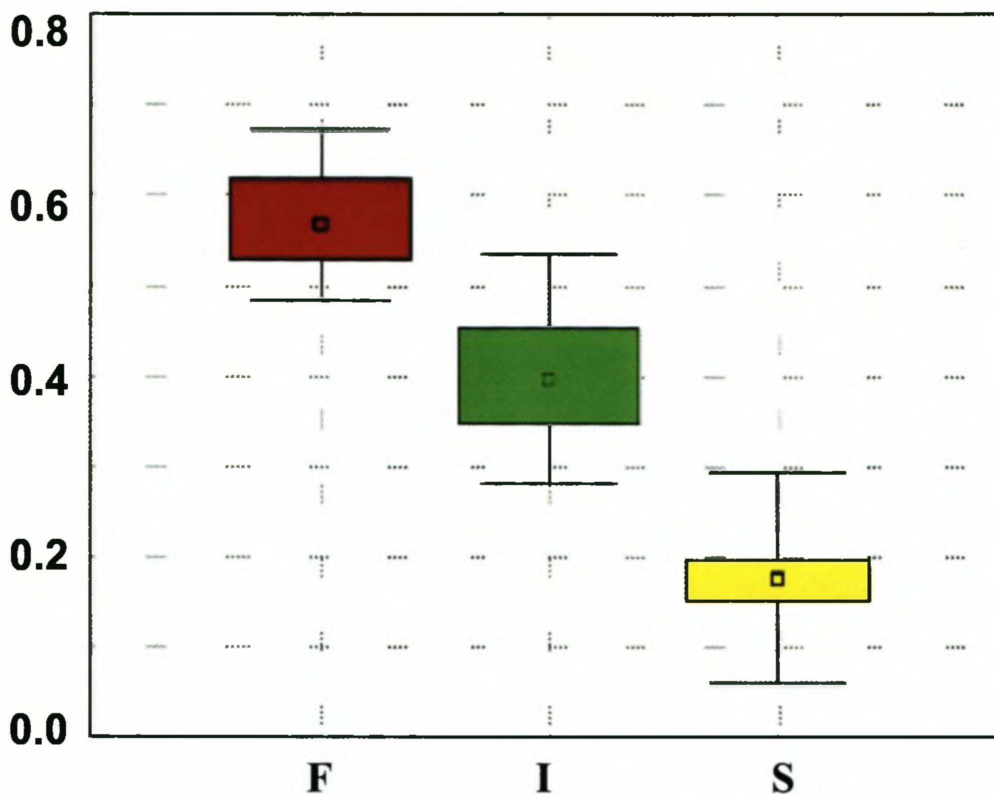

Figure 2. First order elimination rate constants divide the group into three eliminator subtypes: F=fast, I=Intermediate, S=Slow

Furthermore, in attempting to sub-divide the three groups according to their 13 allelic subgroups a clear trimodal pattern once again was demonstrated. (Figure 3). Linearity was markedly absent and therefore did not support the notion of the existence of more than three phenotypic groups when tested with INH as substrate.

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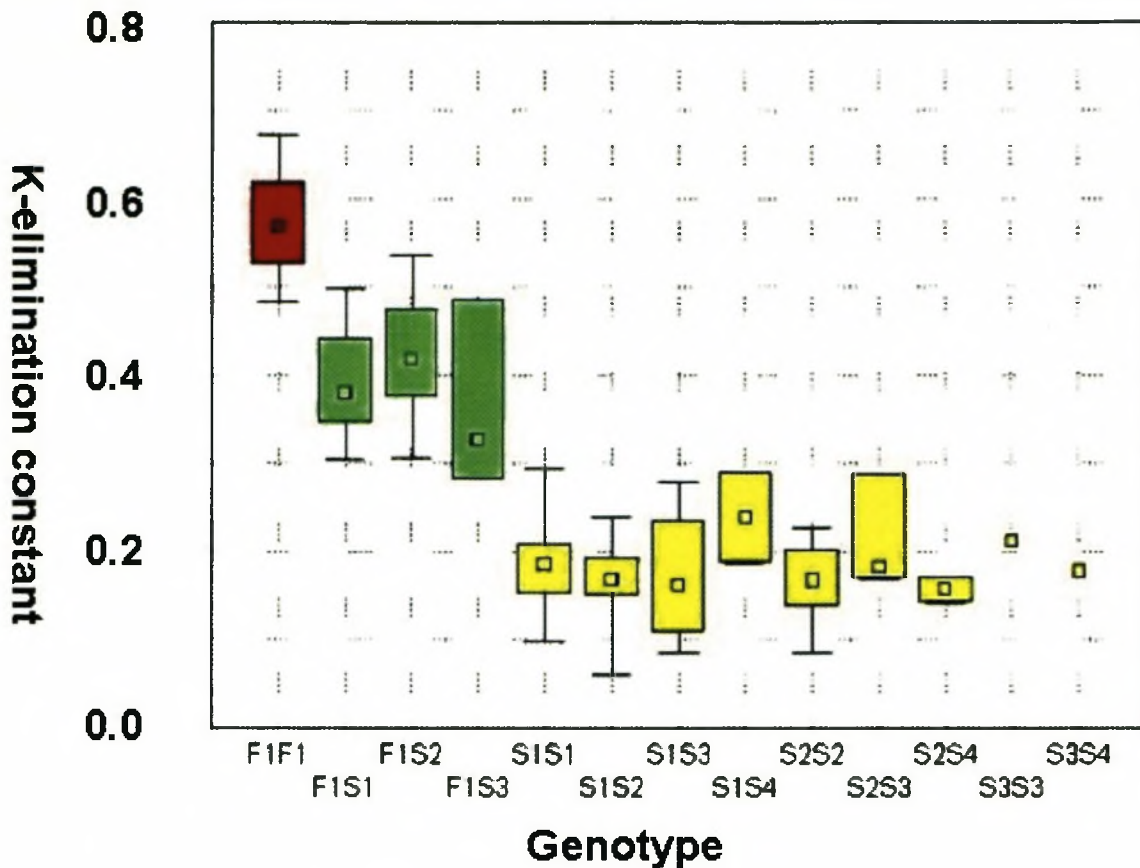

Figure 3. First order elimination rate constants of all the 13 different genotypes demonstrating trimodality.

I. DISTRIBUTION FREQUENCY CHANGE IN SUBGROUPS

CHANGE IN PHENOTYPE FREQUENCIES

Comparing the frequencies of the acetylator subtypes of the studygroup and the controls, it was clear that the cases contained a significantly greater proportion of slow acetylators than the controls (p=0.008). The difference in the fast and intermediate groups was not significant. (Figures 4&5).

STUDYGROUP (n=139)	CONTROLS (n=114)
Fast=22(16%)	Fast=26(22.8%)
Inermediate=55(40%)	Intermediate=54(47,4%)
Slow=62(45%)	Slow=34(29,8%)

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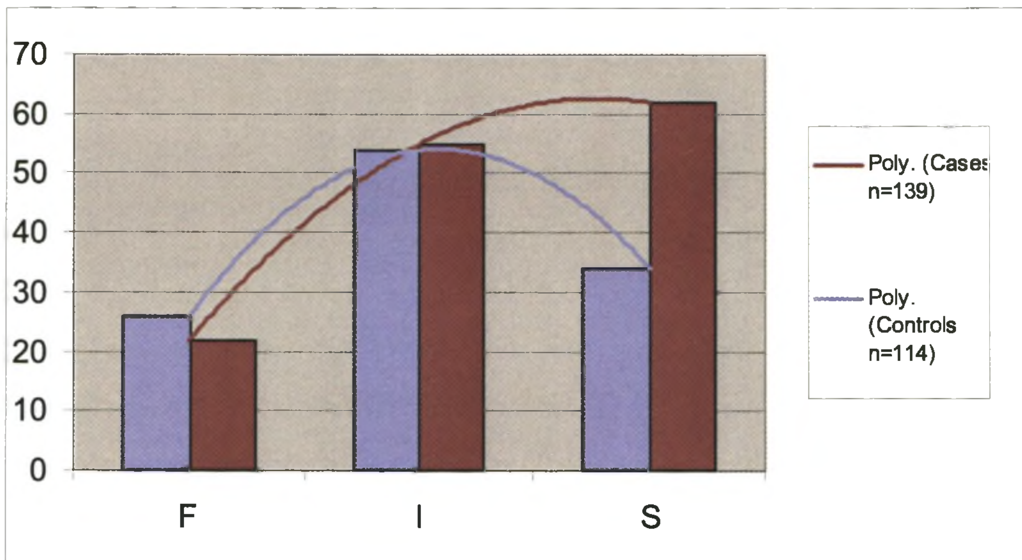

Figure 4. Second degree polynomial trend lines using the absolute numbers of cases and controls (Phenotypes).

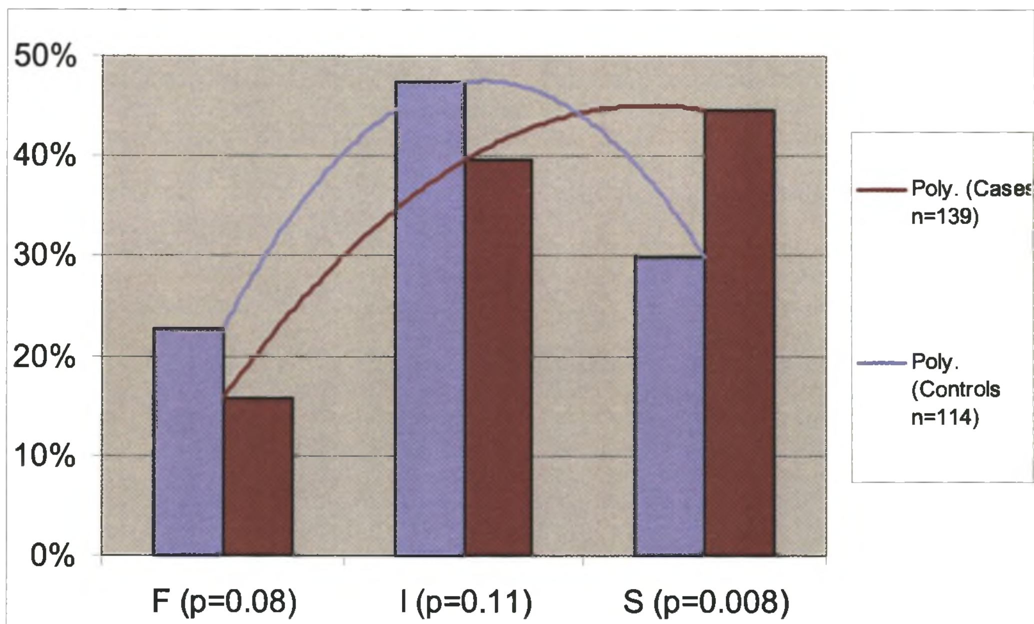

Figure 5. Second degree polynomial trend lines using percentages and determining p-values for differences between groups (Phenotypes).

CHANGE IN ALLELE FREQUENCIES

Allele frequencies were determined by counting alleles. The control group consisted of 106 fast and 122 slow alleles (total = 228) and the cases consisted of 99 fast and 179 slow alleles (total = 278). When expressed as percentages, a significant decrease in the fast alleles and an equally significant rise in the slow alleles in the cataract group was evident (p=0.0066). Figure 6

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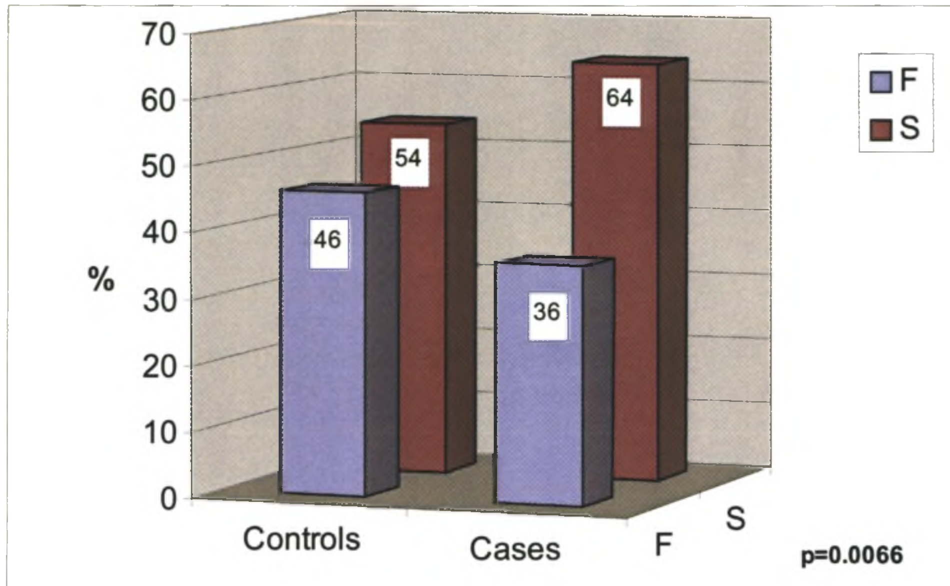

Figure 6. A comparison of allele frequencies between the two groups.

IV. ODDS RATIOS

Odds ratios were determined to assess the risk ratio of cases in the cataract group compared to the control group in a trimodal fashion. This was determined both for the phenotypes and with the aid of the allele distribution pattern in accordance with the Hardy-Weinberg principles. (Tables 7 & 8).

Odds ratios increased when comparing groups with progressively more slow alleles and slow phenotypes. (Figure 7).

Table 7. Odds ratios for the pheno-/genotypes:

Cases	Controls		Cases	Controls		Cases	Controls	
39.57	47.37	I	44.60	29.82	S	44.60	29.82	S
15.83	22.81	F	39.57	47.37	I	15.83	22.81	F
	OR	1.20		OR	1.79		OR	2.16
CI	0.57	2.59	CI	0.96	3.35	CI	0.98	4.77

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Table 8. Odds ratio's for allele frequencies (Hardy-Weinberg):

Cases	Controls		Cases	Controls		Cases	Controls	
45.8	49.76	I	41.5	28.62	S	41.5	28.62	S
12.7	21.62	F	45.8	49.76	I	12.7	21.62	F
	OR	1.57		OR	1.57		OR	2.44
CI	0.70	3.50	CI	0.89	4.05	CI	1.05	5.66

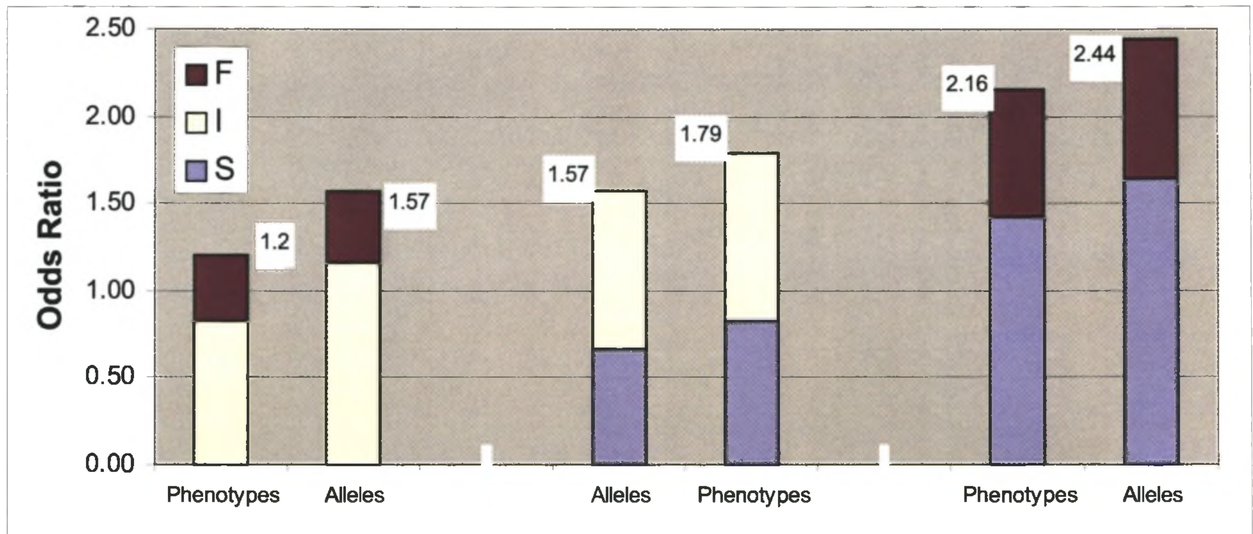

Figure 7. Increasing ODDS RATIO above 1 with progressively more slow acetylator alleles as well as slow phenotypes.

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Phenotypes							Allele Distribution						
Cases %	Controls %	Sub-group	OR	LCL	UCL	p-value	Cases %	Controls %	Sub-group	OR	LCL	UCL	p-value
44.6	29.82	S					41.5	28.62	S				
15.83	22.81	F					12.7	21.62	F				
			2.16	0.98	4.77	**0.028**				**2.44**	1.05	5.66	**0.019**
44.6	29.82	S					41.5	28.62	S				
55.4	70.18	F+I					58.5	71.38	F+I				
			1.89	1.06	3.38	**0.016**				**1.77**	0.98	3.19	**0.029**
44.6	29.82	S					41.5	28.62	S				
39.57	47.37	I					45.8	49.76	I				
			1.79	0.96	3.35	**0.034**				**1.57**	0.84	2.93	**0.078**
84.17	77.19	S+I					87.3	78.38	S+I				
15.83	22.81	F					12.7	21.62	F				
			1.57	0.77	3.20	**0.107**				**1.9**	0.89	4.05	**0.048**
60.43	52.63	F+S					53.2	50.24	F+S				
39.57	47.37	I					46.8	49.76	I				
			1.37	0.78	2.40	**0.136**				**1.17**	0.67	2.04	**0.290**
39.57	47.37	I					45.8	49.76	I				
15.83	22.81	F					12.7	21.62	F				
			1.2	0.56	2.59	**0.321**				**1.57**	0.70	3.50	**0.135**

Table 9. Odds ratios (with confidence intervals and p-values for the trend) in subgroup comparison for both phenotypes and allele frequencies.

Figures 7 and 8, utilising the data from Table 9, illustrate the progressive strength of the relationship (ODDS RATIO) between age related cataract cases and the slow acetylator state, when comparing the different subgroups' contribution to the number of slow alleles and hence odds ratios. The strongest relationship appears when the slow and fast subgroups are compared in respect of allele frequencies (OR =2.44, CI=1.05-5.66, p=0.019) and for phenotype differences (OR=2.16,CI =.98-4.77, p=0.028).

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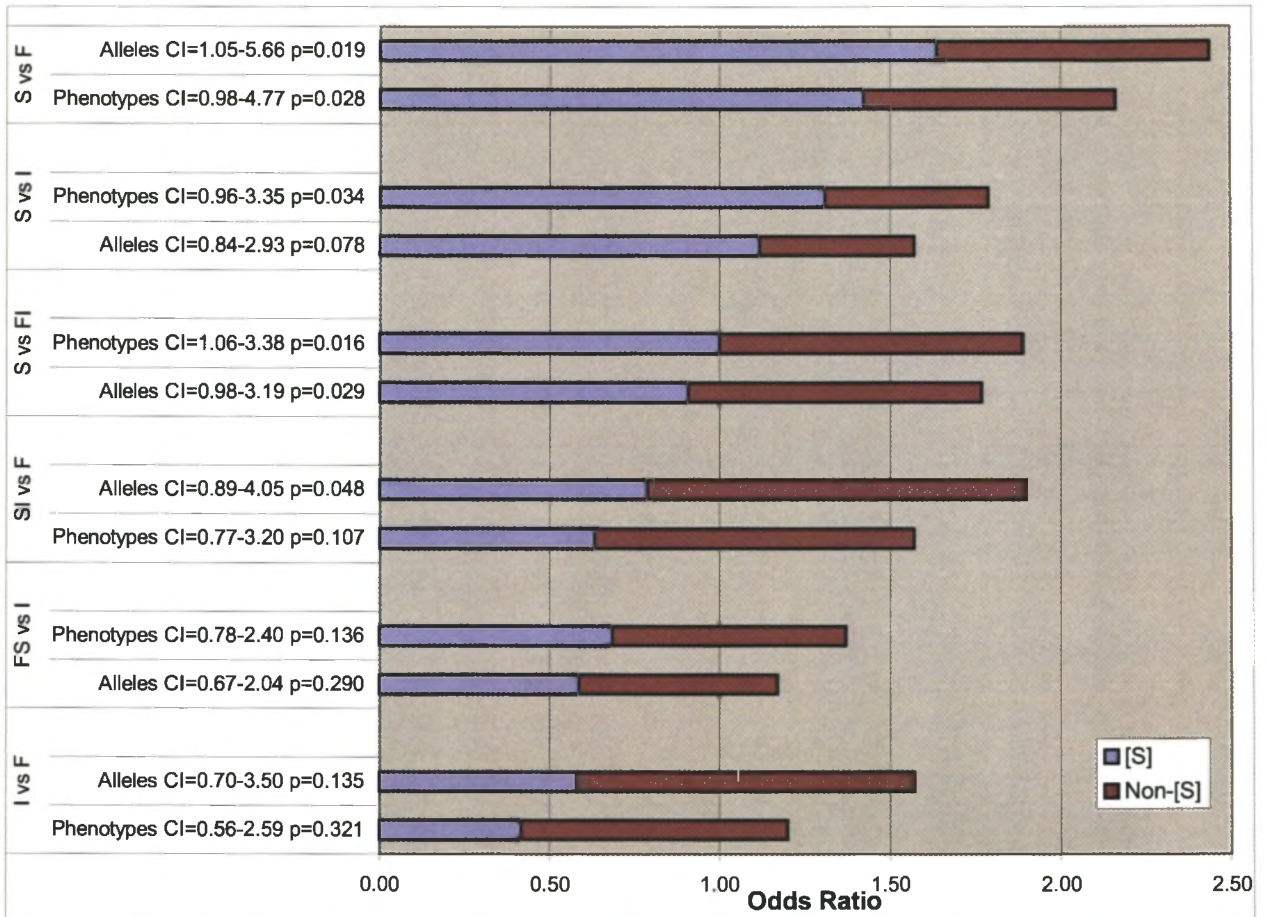

Figure 8. Horizontal bargraph depicting increasing contribution of slow alleles(blue) to the increasing odds ratios with matching progressive significance.

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D. DISCUSSION

In the present study, it was confirmed that the slow acetylator phenotype was significantly more prevalent in the cataract population than in the control group. The number of slow alleles were also significantly more in the cataract group than in the control group of subjects. This is the first time, to our knowledge that such an association between a degenerative eye condition and slow acetylator status has been described. This association sets noteworthy standards because in both the control and the study groups total concordance exists between the pheno- and genotypes. This association was furthermore confirmed by using both a bimodal and a trimodal model.

Several other diseases have been associated with the slow acetylator state. The best documented is urinary bladder cancer[32,33,34,35]. Others include Familial Parkinson's disease[36], epilepsy[37], Gilbert's syndrome[38], endometriosis[39], renal cell carcinoma[40], malignant mesothelioma[41,42], esophageal carcinoma[43], asthma (isocyanate-induced)[44], allergic/atopic disease[45,46], Stevens-Johnson syndrome[47], systemic lupus erythematosus and systemic sclerosis[48]. In the afore-mentioned auto-immune diseases it is postulated that non-acetylated xenobiotics may accumulate and then subsequently become metabolized by other enzymes into reactive intermediates. This enhanced formation of reactive metabolites could alter self-proteins presented to the immune system thus stimulating autoreactive T cells, which induce autoimmunity.

PATHOGENESIS

It is proposed that acetylation aids in the prevention of human lens protein condensation (Figure 9). Possible molecular pathogenetic mechanisms of cataract formation in patients with the slow acetylator pheno-and genotype may be broadly divided into endogenous and exogenous pathways. Support is also gleamed from the recent discovery of muscarinic receptors in the lens epithelial cells and the possible role of acetylcholine in cataractogenesis.

ENDOGENOUS PATHWAYS:

DEVELOPMENTAL PROCESS: α-CRYSTALLIN

Before considering the role of the NAT enzyme system in the lens any further it might be enlightening to consider a few physiological properties of the lens confirming that it is one of the most unique organs in the human body. The vertebrate eye lens is a highly specialized organ whose sole function is to carry out proper refraction of incident light beams in order to ensure visual acuity. It is completely devoid of both blood vessels and nerve supply, and gets its nourishment from the surrounding fluid, the aqueous humor. It is an elegantly simple tissue made of only two types of cells: epithelial cells, which have not yet completely differentiated and not yet elaborated the major gene products, and fiber cells, in which these processes have been initiated or even completed.[49] The lens fiber cells comprise more than 90% of the lens bulk. Between these fiber cells a unique

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and extensive system of iow-resistance gap junctions exists.[50] Unlike all other organs, the lens continues growing throughout the lifespan of the organism[51]. The solid mass of the lens is uniquely about 98% protein.[52] α-Crystallin is one of the major vertebrate lens proteins. Due to its long life in the eye lens, α-crystallin is one of the best-studied proteins with respect to post-translational modifications, including age-induced alterations. α-Crystallin is a rather unique eukaryotic protein in that its N-terminal methionine residue, becomes acetylated during peptide growth, and remains in the polypeptide chain as such. The only other hitherto known N-terminal acetylated methionine residue occurs in the coat protein of turnip yellow mosaic virus particles and in tropomysosin from rabbit muscle.[53] Generally during aging of proteins, there is the general phenomenon of loss of material, which implies an imbalance between biosynthesis and breakdown. The human eye lens, however, forms an exception to that rule since the intra-cellular protein level is virtually maintained during the whole life span of the person. Acetylated proteins present in the nucleus of the adult lens were synthesized during fetal life[54]. It is conceivable therefore that an inherited trait such as the slow acetylator state may influence the quality of the α-crystallins in the lens for life and then only at the point of aging, express the altered proteins which manifests clinically as cataract. This most likely takes place earlier than in those subjects who inherit the wild type gene and phenotypically express the fast acetylator state.

AGING PROCESS: GLYCATION

Postsynthetic modifications of lens crystallins such as nonenzymatic glycation is believed to play a role in cataractogenesis by leading to conformational changes in the crystallins resulting in protein condensation[55]. Glycation involves condensation of a sugar aldehyde or ketone with a free amino group, resulting in the rapid formation of a Schiff base, eventually leading to protein insolubilization and brown, fluorescent products[56]. Glycation is an irreversible process whose rate is dependent on plasma glucose concentration. It is well accepted that aspirin as acetyl salicylic acid (ASA) inhibits glycation by the prior acetylation of amino groups on protein[57] and this could protect the lens proteins against chemical insults resulting in cataract[58]. Acetylation of circulatory proteins such as hemoglobin and albumin inhibits glycation of these molecules[59]. Because glycation of proteins is an important part of the aging process, once proteins have been adequately acetylated they are relatively protected against glycation and hence aging. Slow acetylators have a disadvantage in this respect and their lens protein is therefore probably more prone to alteration, denaturation and aggregation and at an earlier age than the lens protein of fast acetylators.

EXOGENOUS PATHWAYS

ULTRAVIOLET RADIATION: KYNURENINE

The exact role of the arylamine N-acetyltransferases in the lens or in any other tissue still remains unknown although their potential usefulness in metabolism of endogenous amines is obvious. Very little research has been done on the presence of the N-Acetyltransferase (NAT) system in the vertebrate eye. Gaudet et al [60] identified and partially characterized NAT activity in the bovine lens. They suggest that the NAT activity in the

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lens could play a major role in the detoxification of exogenous amines such as ocular drugs. They also raised the possibility of arylamine NAT involvement in the acetylation of endogenous biogenic amines naturally found in the lens such as kynurenine. This tryptophan metabolite is thought to protect the eye from UV-induced photo damage. Kynurenine has two amine moieties, one being an arylamine and the other being an arylakylamine in structure. If arylamine NAT activity in the lens is involved in the N-acetylation of kynurenine or its metabolites, it could protect the lens from extra cellular insult and UV-induced photo damage. This protective ability is conceivably impaired in patients who are slow acetylators.

DRUG ADMINISTRATION: XENOBIOTICS

When NAT 2 acetylates the oxygen atoms of certain hydroxylamines (which can arise from amines by cytochrome P450-dependant oxidation), an acetoxy ester is produced that may be chemically unstable. Cleavage of the N-O bond thus yields an electrophilic nitrenium ion that can bind to nucleophilic sites on intracellular macromolecules, such as DNA bases or proteins. This binding can ultimately result in genotoxicity or cytotoxicity.[61] Any effect upon lens protein therefore is most likely strongly dependent on exposure to arylamine and hydrazine substances (xenobiotics). Adequate detoxification of xenobiotics, by means of the acetylation pathway, therefore prevents protein damage and condensation. In the slow acetylator subgroup this protective pathway functions less optimally than in the fast subgroup, causing an increase in the risk of lens changes.

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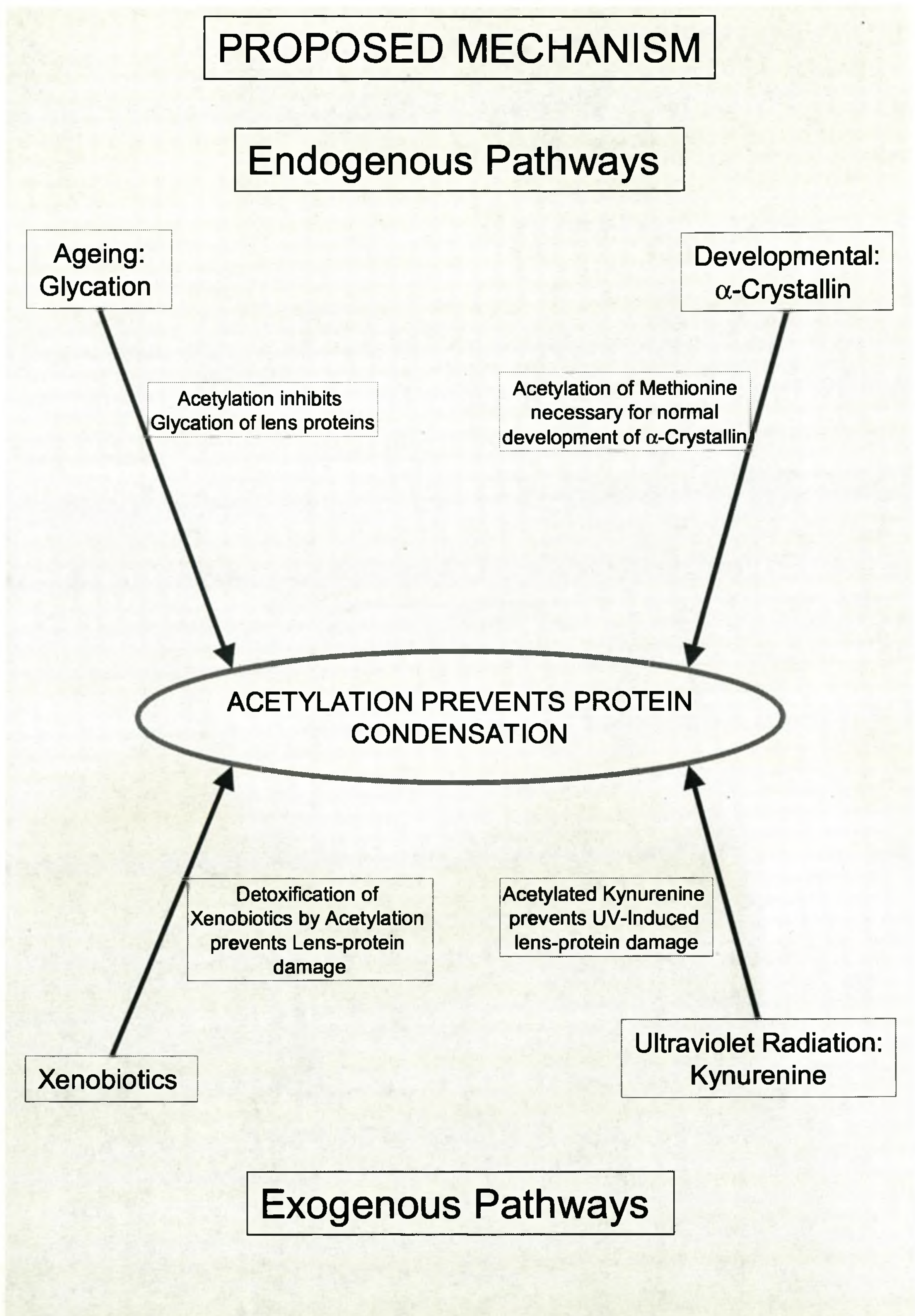

Figure 9. Proposed Mechanisms by which Acetylation prevents lens-protein degradation and condensation

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N-ACETYLTRANSFERASE (NAT) AND CHOLINE ACETYLTRANSFERASE (CAT) – IS ACETYL COENZYME-A THE COMMON DENOMINATOR AND HENCE TO BLAME?

Miotic agents , and particularly long-acting cholinesterase inhibitors (ChEIs), such as echothiophate, when used for extended periods in the treatment of glaucoma, or accommodative estropia, have the potential to cause tiny anterior subcapsular vacuoles and, occasionally, more advanced anterior and posterior subcapsular lens opacities. Cessation of medication may halt , retard, and occasionally reverse their progression[62]. However, when a combination of atropine and echothiophate are administered topically to the eye (in a monkey model), cataractogenesis is either prevented entirely, or is delayed and of lesser severity[63]. The reason why atropine ameliorates the degenerative process is not known. A mechanical cause, due to atropine induced inhibition of accommodation, has been ruled out in animal studies. It is possible that the pathological changes are due either to a biochemically mediated action, or to a toxic effect attributable to echothiophate, although the possible mechanisms by which the ChEIs may induce these changes, remain unclear.

It is also possible that a local increase in the concentrations of acetylcholine, due to the action of the ChEI, may play a role. The lens capsule contains a very high concentration of true acetyl-cholinesterase enzymes (as opposed to pseudocholinesterase) with characteristics similar to those found in neural tissues[64]. This fact, taken in association with the protective effect of atropine on ChEI induced pathology, would seem to imply that the lens possesses functional muscarinic cholinergic receptors, or, alternatively, that the cholinesterase enzymes modify the aqueous and / or vitreous humors in such a way as to render them cataractogenic.

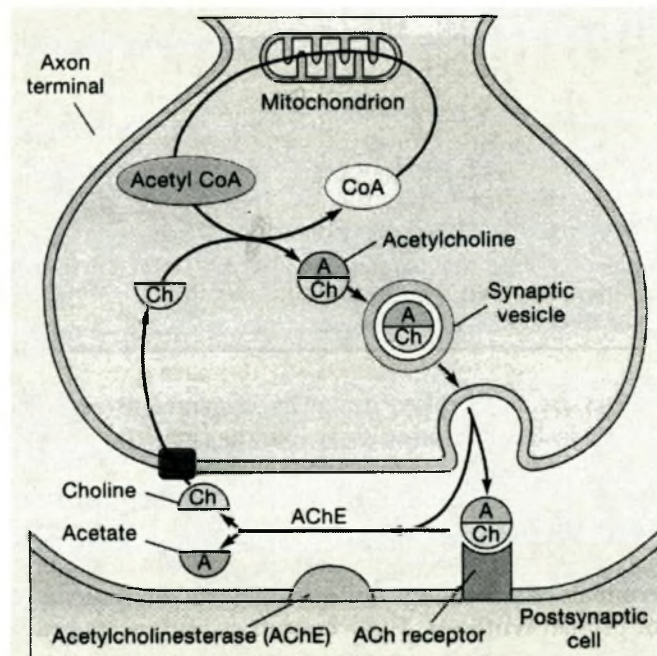

Figure 10. Synthesis and recycling of acetylcholine at the synaps.

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Acetyl-choline (ACh) is one of the main neurotransmitters in the nervous system and is synthesized from choline and acetyl co-enzyme A (acetyl CoA). Choline is a small molecule and is present in all cell membranes, whilst acetyl CoA is the ubiquitous metabolic intermediate that links glycolysis to the citric acid cycle. The synthesis of ACh from these precursors is a relatively uncomplicated process that takes place in the axon terminal (Figure 10 refers). Once released into the synaptic cleft, ACh is rapidly degraded to choline and acetate by ChE enzymes located in the extracellular matrix, and in the membrane of the post-synaptic cell. The choline that is released is transported into the pre-synaptic neuron where it is re-cycled in the synthesis of acetyl-choline, which is stored in synaptic vesicles prior to release[65]. Synthesis of Ach is catalysed by the enzyme choline acetyl-transferase (CAT) [66]. Although this enzyme (CAT) differs from N-acetyltransferase (NAT) in some respects, both mediate acetylator function and transfer acetate to acceptor molecules.

It has recently been established that high concentrations of calcium in the epithelial cells of the lens are cytotoxic and hence conceivably cataractogenic[67]. It has also been shown that acetylcholine induces a concentration dependent increase in peak-amplitude cytosolic calcium in these (epithelial) cells.

The foregoing observations taken together would appear to create a dilemma. High acetylator function via CAT and/or retardation of ACh catalysis, generating high levels of acetylcholine, have the potential to be lentotoxic (via induction of high levels of intracellular calcium), whilst compromised (low) acetylator function, via slow NAT acetylator-status, also mediates lens opacification and cataractogenesis. Consequently, it is clear that, although acetylation function has intriguing correlations and associations with lens degeneration, the mechanisms involved in the pathogenesis are complex and multifaceted, and need to be investigated further.

TRIMODALITY VERSUS BIMODALITY

As indicated elsewhere, N-acetylation in humans is mediated by two distinct cytosolic enzyme systems characterised by different physico-chemical properties and substrate specificities. These two systems are denoted by N-acetyltransferase type 1 (NAT 1) and N-acetyltransferase type 2 (NAT 2). Although the NAT 1-system has recently been shown to be polymorphic in humans, the extent to which the polymorphism manifests in the phenotype does not cause a marked difference in the rate at which the different subgroups metabolise substrate[68]. In stark contrast, the NAT 2-system is highly polymorphic in the sense that, 1), three distinct acetylator phenotypes can be distinguished if an appropriate marker substrate (e.g., isoniazid) is used, and, 2), the extent to which the polymorphism manifests in the phenotype does cause a marked difference in the rate at which the different subgroups metabolise substrate.

The NAT 2- system is relevant to this study. Since a number of gene alleles code for fast and for slow acetylation characteristics, for convenience and simplicity the genotypes corresponding with the phenotypes are denoted by FF, FS, and SS for homozygotic fast, heterozygotic intermediate and homozygotic slow, respectively, F and S being generic for a number of slow and fast alleles, respectively[69,70].

The distribution of polymorphic NAT 2 genes, and their corresponding phenotypes, have been well-studied in the Cape Coloured population[71]. This population has been shown to segregate into three distinct phenotypic subgroups when isoniazid is used as marker substrate, in accordance with the foregoing discussion. Furthermore, the three subtypes are well-represented in the population, as (approximated values) follows: fast

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(FF) – 20%; intermediate (FS) – 50%; slow (SS) – 30%. As such, this population is ideally suited to studies investigating correlations between disease states (e.g., lens opacification) and acetylator characteristics.

In this study a clear trimodal distribution of acetylator genotypes / phenotypes could be demonstrated (Figures 2 and 3) that is biased in the direction of slow acetylator characteristics, i.e., slow acetylators were over-represented in our population comprised of individuals presenting with age-related cataracts. The validity and significance of the bias could be confirmed statistically with a high degree of sophistication, since each allele could be determined by molecular analysis of DNA, and concordance between genotype and phenotype could be confirmed in all instances.

GENE DISTRIBUTION IN ETHNIC GROUPS

To date, 26 different NAT 2 alleles have been identified in human populations. *NAT 2 *4* is considered the wild-type high activity (rapid or fast acetylator) allele. However *NAT 2 *4* is not the most common allele in many populations. Striking differences in frequency of slow acetylator alleles exist among different ethnic groups[72]. The following table illustrates this fact.

Average Bimodal distribution of Acetylator status (%)

USA	65,0 (Slow)	35,0 (Fast)
Europe	48,4 (Slow)	51,6 (Fast)
East Asia	12,0 (Slow)	88,0 (Fast)
Middle East	63,6 (Slow)	36.4 (Fast)
Africa	11,0 (Slow)	89,0 (Fast)

The bimodal distribution in the USA is on par with the Middle East countries. The Caucasians in Europe have an almost equal distribution between slow and rapid acetylators. East Asia and Africa compare favorably with almost 90% of the population manifesting as rapid acetylators.

Very few trimodal population studies exist:

USA	40,0 (Slow)	40,0 (Intermediate)	20,0 (Fast)
England	55,0 (Slow)	35,0 (Intermediate)	10,0 (Fast)

Analysis of DNA, and classification of genotypes, in accordance with a trimodal pattern of distribution of genotypes/phenotypes, allows direct determination of all the alleles constituting the trial population. Direct determination of alleles allows more accurate comparison with the distribution of alleles in the comparator population, better indicating the magnitude and direction of skewing. Direct determination of alleles is set to become the norm in future epidemiological studies of this kind.

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E. CONCLUSION

The question quite rightly arises as to the clinical significance of the association of cataract with the slow acetylator state. In South Africa it is estimated that a cataract backlog of 155,000 cases currently exists (data from Bureau for the Prevention of Blindness). Lens extraction is the most frequently performed surgical procedure in the world and the costs associated with lens problems comprise the largest line item in the Medicare budget in the United Kingdom[73]. In the USA more than two million lens extractions are performed annually with the attendant significant health care costs (US $ 5 billion)[74]. In South Africa the challenge of addressing the cataract backlog problem is daunting and estimates to achieve this exceed R20 milion annually.

The ability to accurately classify a patient genotypically and then confirming it phenotypically early in life, now exists. This knowledge may be used in health counseling. Should a person be a known slow acetylator, additional preventative precautions may be indicated such as the prevention of UV-exposure to the eye and caution with the ingestion of xenobiotics that are homo- and heterocyclic arylamines or hydrazines e.g. caffeine, commercial dyes, food preservatives and drugs such as INH and sulphonamides.

The search for etiological mechanisms of cataract in humans continues. Genetic mechanisms that may contribute to modification and condensation of lens protein should be considered with greater enthusiasm.

This study compels us to submit that the slow acetylator pheno- and genotype may be regarded as a risk indicator for age related cataract.

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APPENDIX

METHODOLOGY OF DETERMINING GENOTYPE BY MEANS OF POLYMERASE CHAIN REACTION (PCR) AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

METHODOLOGY - POLYMERASE CHAIN REACTION

In this study peripheral whole blood was collected in EDTA tubes from each patient and stored at –20°C until required for DNA analysis. Genomic DNA was isolated from peripheral white blood cells and used in the analysis of the alleles for the N-acetyltransferase enzyme (NAT2) using the Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) techniques.

Restriction fragments were resolved by gel-electrophoresis using Metaphor agarose, after which the gels were stained with ethidium bromide to delineate the specific NAT2 allelic variants. Results were scored according to the nomenclature of Vatsis et al., (1995).[1] Our analysis focused on those NAT2 alleles known to cause a corresponding phenotypic change on the activity of the NAT2 protein, in order to obtain a genotype vs phenotype correlation for each patient in the study. All chemicals and reagents used were of the purest grade commercially available.

1 DNA PURIFICATION

DNA was extracted from whole blood using a modification of the "salting-out" method of Miller et al (1988).[2] One volume of whole blood was diluted with five to six volumes of ice cold Cell Lysis Buffer (320 mM Sucrose; 1% Triton X-100, 5 mM $MgCl_2.6H_2O$, 10 mM Tris-HCl, pH 7,6). The tubes were mixed vigorously to lyse the cells, and then centrifuged at 3000 rpm at 4°C for fifteen minutes. The supernatant was discarded and the pellet resuspended in 10 ml Nuclei Lyses Buffer (400 mM NaCl; 10 mM Tris-HCl, pH 8,2; 2 mM EDTA, pH 8,2). One ml of a 10% Sodium Dodecylsulphate (SDS) solution was added (gently mixed), followed by adding Proteinase K solution (10 mg/ml), to a final concentration of 100 µg/ml; the tubes were then incubated overnight at 37°C, with gentle shaking to aid in the dissolution of the particulate matter.

After digestion, 3 ml of saturated NaCl (> 6 M) was added to each tube, mixed thoroughly but gently, and again centrifuged at 3000 rpm for fifteen minutes at 4°C. The supernatant was then transferred to a clean tube; 2 - 3 volumes of room temperature absolute ethanol (100%) was added, and the DNA was allowed to float to the top of the solution. The DNA was recovered using a sealed Pasteur pipet and washed with a 70% ethanol solution (500 µl). This supernatant was discarded, the DNA pellet dried at 37°C for ten minutes, and then redesolved in

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one ml of TE buffer (10 mM Tris-HCl, pH 7,5; 2 mM EDTA, pH 7,5). The DNA concentration was determined using the Beer Lambert Law by reading the absorbance of each sample DNA at 260 nm.

2 NAT2 SEQUENCE AMPLIFICATION

The PCR amplification licensed to Perkin Elmer of DNA is an in vitro technique for the primer directed-enzymatic amplification of specific DNA sequences. The specificity of amplification is based on two oligonucleotide primers (Nat-Hu 14 and Nat-Hu 16) which flank the DNA segment (NAT2) to be amplified, resulting in the exponential accumulation of the specific target fragment (the NAT2 gene sequence) by selective temperature cycling.

Standard PCR reaction mixture contains:

(1) 1 x PCR buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl, 0,01% gelatin (w/v), 0,01% Triton X-100).

(2) 2 mM $MgCl_2$; (this $MgCl_2$ concentration was optimized for the reaction).

(3) 0,2 mM dNTP (deoxy - Nucleotide Tri-phosphate) mixture, where N = mixture of Adenine, Guanine, Citosine and Thymine.

(4) The primer-pair consisting of Nat-Hu 14 and Nat-Hu 16, added to a final concentration of 0,5 µM each.

Heat-stable DNA polymerase (94 kDa) of the archebacterium Thermus aquaticus (Taq polymerase I) was used to a final concentration of 1 unit per reaction. DNA (100-250 ng) template was used per PCR reaction. The parameters for amplification consists of sequential steps of selected temperature cycling. In the case of NAT2 amplification, a 1000 bp sequence of the gene was selectively amplified using the primer pair Nat-Hu 14 and Nat-Hu 16.

The standard PCR program consists of: -

- **1 cycle of** 94°C for 4 minutes (denaturation).
- **30 cycles of** 94°C for 45 seconds (denaturation),
  - 56°C for 60 seconds (annealing), and
  - 72°C for 80 seconds (polymerization).
- **1 cycle of** 72°C for 900 seconds (final polymerization).

In order to test for the specificity of amplification a one tenth volume of the reaction mixture (10 µl) was supplemented with 1-2 µl of a 6x DNA loading buffer [containing 0,25% Bromophenol Blue, 0,15% Orange G and 14% Ficoll Type 400 (Pharmacia)]. This was analyzed by horizontal gel electrophoresis on a 0,7% agarose gel (Seakem, ME agarose, FMC, Rockland, USA) of dimensions 100 mm x 67 mm x 5 mm, using 1x TBE (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA) as running buffer. A current of 5 volts per centimeter (V/cm) was maintained for 45 minutes. Subsequently, the gel was stained by shaking it for 20 minutes at room temperature in a 1x TBE buffer solution containing 0,5 µg Ethidium Bromide per ml. A positive PCR product of a 1000 bp was observed and used in the subsequent Restriction Enzyme analysis for the NAT2-specific alleles.

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3. RESTRICTION ENZYME ANALYSIS

Aliquots of the amplification product (1000 bp fragment) were selectively cleaved with the following **restriction enzymes:**

MspI	Moraxella species
KpnI	Klebsiella pneumonia
BamHI	Bacillus amylobiquefaciens
TaqI	Thermus aquanticus
FokI	Flavobacterium okeanokoites
DdeI	Desulfovibrio desulfuricans

These enzymes cleave at specific nucleotide positions, as indicated below: -

MspI cleaves at	191 (G $\rightarrow$ A)
	434 (A $\rightarrow$ C)
KpnI cleaves at	481 (C $\rightarrow$ T)
BamHI cleaves at	857 (G $\rightarrow$ A)
TaqI cleaves at	590 (G $\rightarrow$ A)

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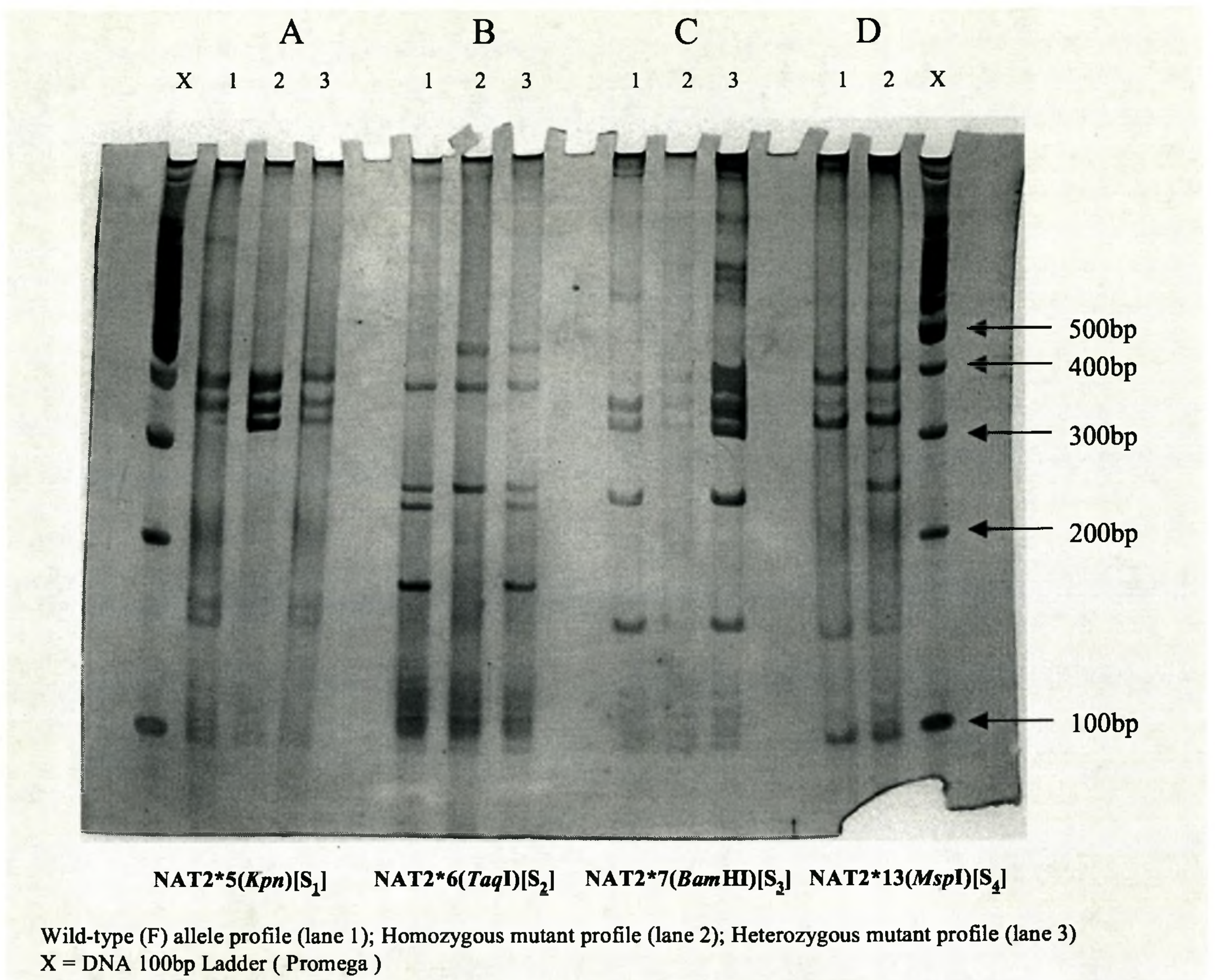

Figure 1. Photo of restriction enzyme profile gel for the *NAT 2* Mutant Alleles. This represents examples of real cases generated in our laboratory during this study.

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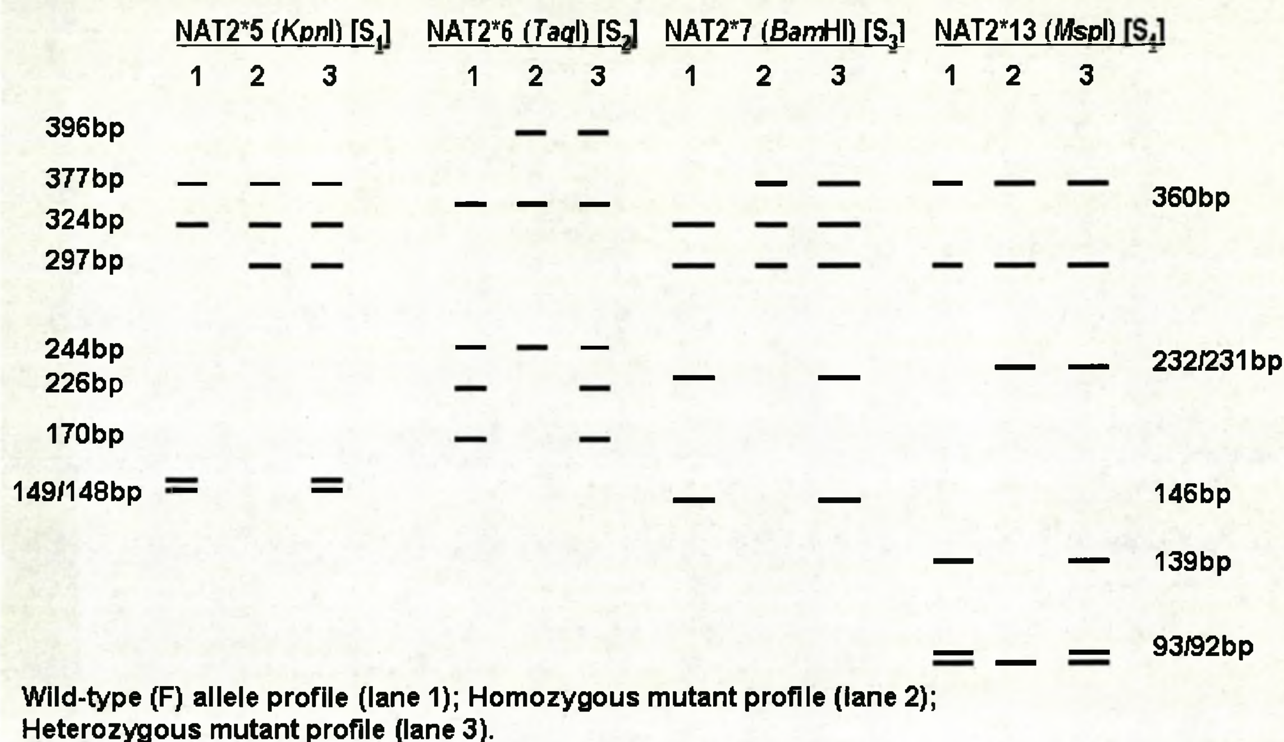

Figure 2. A graphic representation of the restriction enzyme profiles for the *NAT2* Mutant Alleles as scored by this method.

This selective restriction of the 1000 bp fragment is used to identify specific allelic variations of NAT2 at the above options. An aliquot (20 µl) of the PCR product was digested with 10 units of each enzyme in all cases. Roche Diagnostics supplied all enzymes, and digestions were performed according to the manufacturer's specifications. Digestions were incubated at 37°C for 6 hours (or overnight), except in the case of the Taq1 digestions, which were incubated at 65°C for six hours. The (BamHI, KpnI and MspI) digested volume was again supplemented with 2 µl of 6x DNA Loading Buffer. The restriction enzyme digested fragments were resolved by horizontal gel electrophoresis (dimensions of the gel were 100 mm x 67 mm x 5 mm) in 2,5% Metaphor Agarose (FMC BioProducts, Rockland, ME) using 1x TBE buffer (89 mM Tris-HCl, 89 mM boric acid and 2 mM EDTA) as the running buffer. After electrophoresis, the gels were stained with 0.5 µg/ml Ethidium Bromide as above, and then photographed. Results were scored according to the nomenclature of Vatsis et al., (1995).

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4. POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

In order to obtain a better resolution and separation of bands, some of the restriction enzyme digested fragments (Section 1.3) were subjected to a further round of digestion with the enzyme PstI (Providencia stuartii). This enzyme cleaves the 1000 bp NAT fragment into two fragments, of sizes 1050 bp and 1347 bp. This additional cleavage serves to restrict the BamHI, KpnI and MspI RFLP's into smaller fragments that can be resolved on polyacrylamide, thereby increasing the sensitivity of the RFLP analysis. Eight microliters of the 20 µi digested volumes (for BamHI, KpnI and MspI only) were cleaved with 8 units of PstI (in a total volume of 10µl) for 6 hours at 37°C. Digestions were performed according to the manufacturer's specifications. After digestion, 1,5 µl of DNA loading buffer was added and the digested fragments were resolved by 5% non-denaturing PAGE. For 10 ml of gel solution the following components were added in sequence: [1,25 ml of 40% Polyacrylamide stock; 2 ml of 5x TBE buffer; 125 µl of 10% ammonium persulphate (APS); 6,61 ml Milli Q water and 12,5 µl N, N, N', N'-tetramethylethylenediamine (TEMED)]. This is sufficient for 2 gels of 100mm x 60mm x 0,75mm. PAGE gels were run using the Mini Protean II vertical gel apparatus (BIO-RAD, USA) and a 1x TBE buffer (89mM Tris-HCL, 89mM boric acid and 2mM EDTA) as running buffer. A current of 100volts was applied for 1 hour at which time the bromophenol blue dye front had reached the bottom of the gel. After electrophoresis, the gels were silver stained according to the method of Bassam et al., (1991)[3]. Briefly this procedure entails:-

The DNA was fixed by washing the gel with 7,5% acetic acid (preheated to 37°C) at 37°C for 10 minutes. The gel was then rinsed in double distilled water for 3 minutes at 37°C. Rinsing was repeated twice.

A 0,1% silver nitrate solution (preheated to 37°C) containing formaldehyde (final concentration 0,056%), was used for staining the gel at 37°C for 10 minutes.

After silver staining, but prior to band development, the gel was very briefly rinsed in distilled water at room temperature (not more than 10 seconds).

The developing solution consists of a 3% sodium carbonate solution (pre-chilled at –20°C; working temp. ±8–10°C), formaldehyde (final concentration 0,056%) and sodium thiosulphate (final concentration 8µM).

The gel was stained with the above solution for 5–10 minutes at room temperature and observed for band development.

When optimal development (±7 minutes) was obtained, the developing reaction was stopped by placing the gel into an ice-cold solution of 7,5% acetic acid for 5 minutes at room temperature.

In several of the patients, the genotype-phenotype results were discordant; in these cases the sample PCR products were subjected to an additional analysis with the DdeI restriction enzyme to distinguish the NAT2*5A/*5C alleles. These profiles were also resolved via 5% polyacrylamide gel electrophoresis as above.

Finally the gel was rinsed in distilled water for 5 minutes and vacuum packed in a plastic sleeve to form a permanent record of the RFLP profiles of the samples.

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