Université de Montréal

# The Impact of Topical Prostaglandin Analogs on the Biomechanical Properties of the Cornea in Patients with Open Angle Glaucoma

# L'Impact des analogues de prostaglandines topiques sur les propriétés biomécaniques de la cornée chez les patients atteints du glaucome à angle ouvert

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# Résumé


## Justification:

Le glaucome entraîne une perte progressive de la vision causée par la détérioration du nerf optique. Le glaucome est répandu dans le monde et cause la cécité dans environ sept millions de personnes. Le glaucome touche plus de 400 000 Canadiens et sa prévalence augmente avec le vieillissement de la population.[1,2]

Il s'agit d'une maladie chronique surnoise dont les symptômes se manifestent uniquement lors des stades avancés et qui peuvent mener à la cécité. Présentement, le seul moyen possible d'arrêter la progression du glaucome au stade initial est de diminuer la pression intra-oculaire (PIO). Les analogues de prostaglandines (APG) topiques sont fréquemment utilisées comme traitement de première ligne. Cependant, la recherche démontre que cette classe de médicaments peut changer certaines propriétés de la cornée, et possiblement influencer la mesure de la PIO.[3]

## Objectif:

À déterminer si l'utilisation d'APG affecte les propriétés biomécaniques de la cornée. La conclusion sera basée sur l'analyse intégrée des résultats obtenus de l'analyseur Reichert oculaire Réponse (ORA), la tonométrie par applanation de Goldmann (TAG) et la pachymétrie ultrasonographique. Le deuxième objectif potentiel de cette étude est de déterminer la corrélation, le cas échéant, entre les propriétés biomécaniques de la cornée, l'épaisseur de la cornée centrale (ECC) et la PIO chez les patients subissant un traitement d'APG topique.

L'hypothèse principale de cette étude est que l'APG influence les propriétés de la cornée telles que l'épaisseur centrale, l'élasticité et la résistance.

## Patients et méthodes :

Soixante-dix yeux de 35 patients, âgés de 50-85 ans, atteints de glaucome à angle ouvert (GAO) et traités avec APG topique ont été examinés. Seulement les sujets avec une réfraction manifeste entre -6,00 D et +4,25 D ont été inclus. Les critères d'exclusion sont: patients avec n'importe quelle autre maladie de la cornée de l'œil, telles que la dystrophie endothéliale de Fuch's et kératocône, ou tout antécédent de traumatisme ou d'une chirurgie de la cornée, ainsi que le port de lentilles de contact. Nous avons demandé aux patients atteints du glaucome qui ont des paramètres stables et qui utilisent l'APG dans les deux yeux de cesser l'APG dans l'œil moins affecté par la PIO, et de continuer l'utilisation d'APG dans l'œil contralatéral. Le meilleur œil est défini comme celui avec moins de dommage sur le champ visuel (CV) (déviation moyenne (DM), le moins négatif) ou une PIO maximale historique plus basse si la DM est égale ou encore celui avec plus de dommage sur la tomographie par cohérence optique (TCO, Cirrus, CA) ou la tomographie confocale par balayage laser (HRT, Heidelberg, Allemagne). Toutes les mesures ont été prises avant la cessation d'APG et répétées 6 semaines après l'arrêt. Les patients ont ensuite recommencé l'utilisation d'APG et toutes les mesures ont été répétées encore une fois après une période supplémentaire de 6 semaines. Après commencer ou de changer le traitement du glaucome, le patient doit être vu environ 4-6 semaines plus tard pour évaluer l'efficacité de la goutte.[4] Pour cette raison, on

été décidé d'utiliser 6 semaines d'intervalle. Toutes les mesures ont été effectuées à l'institut du glaucome de Montréal par le même technicien, avec le même équipement et à la même heure de la journée. L'œil contralatéral a servi comme œil contrôle pour les analyses statistiques. La tonométrie par applanation de Goldmann a été utilisée pour mesurer la PIO, la pachymétrie ultrasonographique pour mesurer l'ECC, et l'ORA pour mesurer les propriétés biomécaniques de la cornée, incluant l'hystérèse cornéenne (HC).

L'hypothèse de l'absence d'effet de l'arrêt de l'APG sur les propriétés biomécaniques a été examiné par un modèle linéaire à effets mixtes en utilisant le logiciel statistique R. Les effets aléatoires ont été définies à deux niveaux: le patient (niveau 1) et l'œil de chaque patient (niveau 2). Les effets aléatoires ont été ajoutés au modèle pour tenir compte de la variance intra-individuelle. L'âge a également été inclus dans le modèle comme variable. Les contrastes entre les yeux et les temps ont été estimés en utilisant les valeurs p ajustées pour contrôler les taux d'erreur internes de la famille en utilisant multcomp paquet dans R.

## Résultats:

Une augmentation statistiquement significative due l 'HC a été trouvée entre les visites 1 (sur APG) et 2 (aucun APG) dans les yeux de l'étude, avec une moyenne (±erreur standard) des valeurs de 8,98 ± 0,29 mmHg et 10,35 ± 0,29 mmHg, respectivement, correspondant à une augmentation moyenne de 1,37 ± 0,18 mmHg ($p < 0,001$). Une réduction significative de 1,25 ± 0,18 mmHg ($p < 0,001$) a été observée entre les visites 2 et 3, avec une valeur moyenne HC

finale de 9,09 ± 0,29 mmHg. En outre, une différence statistiquement significative entre l'oeil d'étude et le contrôle n'a été observée que lors de la visite 2 (1,01 ± 0,23 mmHg, p <0,001) et non lors des visites 1 et 3.

Une augmentation statistiquement significative du facteur de résistance conréen (FRC) a été trouvée entre les visites 1 et 2 dans les yeux de l'étude, avec des valeurs moyennes de 10,23 ± 0,34 mmHg et 11,71 ± 0,34 mmHg, respectivement. Le FRC a ensuite été réduit de 1,90 ± 0,21 mmHg (p <0,001) entre les visites 2 et 3, avec une valeur moyenne FRC finale de 9,81 ± 0,34 mmHg. Une différence statistiquement significative entre l'oeil d'étude et le contrôle n'a été observée que lors de la visite 2 (1,46 ± 0,23 mmHg, p <0,001).

Une augmentation statistiquement significative de l'ECC a été trouvée entre les visites 1 et 2 dans les yeux de l'étude, avec des valeurs moyennes de 541,83 ± 7,27 µm et 551,91 ± 7,27 µm, respectivement, ce qui correspond à une augmentation moyenne de 10,09 ± 0,94 µm (p <0,001). L'ECC a ensuite diminué de 9,40 ± 0,94 µm (p <0,001) entre les visites 2 et 3, avec une valeur moyenne finale de 542,51 ± 7,27 µm. Une différence entre l'étude et le contrôle des yeux n'a été enregistré que lors de la visite 2 (11,26 ± 1,79 µm, p <0,001).

De même, on a observé une augmentation significative de la PIO entre les visites 1 et 2 dans les yeux de l'étude, avec des valeurs moyennes de 15,37 ± 0,54 mmHg et 18,37 ± 0,54 mmHg, respectivement, ce qui correspond à une augmentation moyenne de 3,0 ± 0,49 mmHg (p <0,001). Une réduction significative de 2,83 ± 0,49 mmHg (p <0,001) a été observée entre les visites 2 et 3, avec une valeur moyenne de la PIO finale de 15,54 ± 0,54 mmHg. L'oeil de

contrôle et d'étude ne différaient que lors de la visite 2 (1,91 ± 0,49 mmHg, p <0,001), ce qui confirme l'efficacité du traitement de l'APG.

Lors de la visite 1, le biais de la PIO (PIOcc - PIO Goldmann) était similaire dans les deux groupes avec des valeurs moyennes de 4,1 ± 0,54 mmHg dans les yeux de contrôles et de 4,8 ± 0,54 mmHg dans les yeux d'études. Lors de la visite 2, après un lavage de 6 semaines d'APG, le biais de la PIO dans l'œil testé a été réduit à 1,6 ± 0,54 mmHg (p <0,001), ce qui signifie que la sous-estimation de la PIO par TAG était significativement moins dans la visite 2 que de la visite 1. La différence en biais PIO moyenne entre l'étude et le contrôle des yeux lors de la visite 2, en revanche, n'a pas atteint la signification statistique (p = 0,124). On a observé une augmentation peu significative de 1,53 ± 0,60 mmHg (p = 0,055) entre les visites 2 et 3 dans les yeux de l'étude, avec une valeur de polarisation finale de la PIO moyenne de 3,10 ± 0,54 mmHg dans les yeux d'études et de 2,8 ± 0,54 mmHg dans les yeux de contrôles.

Nous avons ensuite cherché à déterminer si une faible HC a été associée à un stade de glaucome plus avancé chez nos patients atteints du glaucome à angle ouvert traités avec l'APG. Lorsque l'on considère tous les yeux sur l'APG au moment de la première visite, aucune association n'a été trouvée entre les dommages sur le CV et l'HC.

Cependant, si l'on considère seulement les yeux avec un glaucome plus avancé, une corrélation positive significative a été observée entre la DM et l'HC (B = 0,65, p = 0,003). Une HC inférieure a été associé à une valeur de DM de champ visuelle plus négative et donc plus de dommages liés au glaucome.

**Conclusions :**

Les prostaglandines topiques affectent les propriétés biomécaniques de la cornée. Ils réduisent l'hystérèse cornéenne, le facteur de résistance cornéen et l'épaisseur centrale de la cornée. On doit tenir compte de ces changements lors de l'évaluation des effets d'APG sur la PIO. Plus de recherche devrait être menées pour confirmer nos résultats. De plus, d'autres études pourraient être réalisées en utilisant des médicaments qui diminuent la PIO sans influencer les propriétés biomécaniques de la cornée ou à l'aide de tonomètre dynamique de Pascal ou similaire qui ne dépend pas des propriétés biomécaniques de la cornée. En ce qui concerne l'interaction entre les dommages de glaucome et l'hystérésis de la cornée, nous pouvons conclure qu' une HC inférieure a été associé à une valeur de DM de CV plus négative.

## Mots Clés

glaucome - analogues de prostaglandines - hystérèse cornéenne – l'épaisseur de la cornée centrale - la pression intraoculaire - propriétés biomécaniques de la cornée.

# ABSTRACT


## Rationale:

Glaucoma is a chronic disease that causes a gradual loss of vision due to progressive damage to the optic nerve. It is widespread in the world and causes blindness in about seven million people. In addition, it affects more than 400,000 Canadians and its prevalence is increasing with the aging of the population.[1, 2]

Glaucoma becomes symptomatic only in the more advanced stages of the disease, and currently, the goal of treatment is to halt the progression of the disease by lowering the intra-ocular pressure (IOP). Topical prostaglandin analogs (PGA) are currently the first line treatment, however research has shown that this class of medications may change certain properties of the cornea, and hence the measurement of IOP.[3]

## Aim:

To determine whether the use of topical prostaglandin analogs (PGA) affects the biomechanical properties of the cornea. The conclusion will be based on the integrated analysis of the data collected from the Reichert Ocular Response Analyzer (ORA), Goldmann tonometry and ultrasound pachymetry. The second potential aim of this study is to determine the correlations, if any, between the biomechanical properties of cornea, the Central Corneal Thickness (CCT) and IOP in patients undergoing topical PGA treatment.

The main hypothesis of this study is that the PGA drops influence the properties of the cornea such as central thickness, elasticity and resistance.

## Patients and Methods:

In this study, seventy eyes of 35 patients, aged 50 - 85 years, with open angle glaucoma (OAG) and treated with topical PGA were examined. Only subjects with a manifest refraction between -6.00 D and +4.25 D were included. Exclusion criteria included patients with any other corneal eye disease, such as Fuch's endothelial dystrophy or keratoconus, or any past history of corneal trauma or surgery. Contact lens wearers were also excluded. Patients with stable glaucoma parameters who were using topical PGA in both eyes prior to the start of the study were asked to discontinue the PGA in the best eye and to continue the application of PGA to the contralateral eye. The "best" eye, representing the eye with the least amount of glaucoma-related damage, was selected based on the results of the Humphrey Visual Field (HFA, Carl Zeiss Meditec, Inc., Dublin, CA), Heidelberg Retinal Tomography (HRT II, Heidelberg Engineering GmbH, Heidelberg, Germany), Optical Coherence Tomography (CIRRUS HD-OCT, Carl Zeiss Meditec, Inc., Dublin, CA) and maximum IOP. The contralateral eye served as a control for statistical analyses. Corneal measurements were taken before PGA cessation and repeated 6 weeks after cessation. Patients then restarted the use of PGA and all measurements were repeated once more after an additional 6 weeks. After starting or changing glaucoma treatment the patient should be seen approximately 4-6 weeks later to assess efficacy of the drop.[4] For this reason it was decided to use 6 weeks interval. All measurements were performed at The Montreal Glaucoma Institute by the same trained technician, with the same equipment and at the same time of day. Goldmann applanation tonometry was used to measure the patient's intraocular pressure (IOP),

ultrasound pachymetry was used to measure central corneal thickness (CCT) and the ORA provided the measurements of the corneal biomechanical properties, including corneal hysteresis (CH).

The hypothesis of no effect regarding the discontinuation of PGA on the biomechanical properties was examined by a linear mixed-effect model using the nlme package in R. Random-effects was defined on two levels: the patient (level-1) and the eye within each patient (level-2). Those random-effects were added to the model to account for the intra-individual variance due to the repeated-measure design. Age was also included in the model as a covariate. Contrasts between the eyes and times were estimated using adjusted p-values to control for familywise error rate using multcomp package in R.

## Results:

A statistically significant increase in CH was found between Visit 1 (on PGA) and Visit 2 (no PGA) in the study eyes, with mean (±Standard Error) values of 8.98 ± 0.29 mmHg and 10.35 ± 0.29 mmHg, respectively, corresponding to a mean increase of 1.37 ± 0.18 mmHg ($p < 0.001$). A significant reduction of 1.25 ± 0.18 mmHg ($p < 0.001$) was also observed between Visits 2 and 3, with a final mean CH value of 9.09 ± 0.29 mmHg. In addition, a statistically significant difference between the study and control eyes was only observed at Visit 2 (1.01 ± 0.23 mmHg; $p < 0.001$) and not at Visits 1 and 3.

A statistically significant increase in Corneal Resistance Factor (CRF) was found between Visits 1 and 2 in the study eyes, with mean values of 10.23 ± 0.34 mmHg and 11.71 ± 0.34 mmHg, respectively. CRF was then reduced by 1.90 ±

0.21 mmHg ($p < 0.001$) between Visits 2 and 3, with a final mean CRF value of 9.81 ± 0.34 mmHg. A statistically significant difference between the study and control eyes was only observed at Visit 2 (1.46 ± 0.23 mmHg; $p < 0.001$).

A statistically significant increase in CCT was found between Visits 1 and 2 in the study eyes, with mean values of 541.83 ± 7.27 µm and 551.91 ± 7.27 µm respectively, corresponding to a mean increase of 10.09 ± 0.94 µm ($p < 0.001$). CCT then decreased by 9.40 ± 0.94 µm ($p < 0.001$) between Visits 2 and 3, with a final mean value of 542.51 ± 7.27 µm. A difference between the study and control eyes was only recorded at Visit 2 (11.26 ± 1.79 µm; $p < 0.001$).

Similarly, a significant increase in IOP was observed between Visits 1 and 2 in the study eyes, with mean values of 15.37 ± 0.54 mmHg and 18.37 ± 0.54 mmHg respectively, corresponding to a mean increase of 3.0 ± 0.49 mmHg ($p < 0.001$). A significant reduction of 2.83 ± 0.49 mmHg ($p < 0.001$) was observed between Visits 2 and 3, with a final mean IOP value of 15.54 ± 0.54 mmHg. The control and study eyes only differed at Visit 2 (1.91 ± 0.49 mmHg; $p < 0.001$), confirming the effectiveness of PGA treatment.

At Visit 1, the IOP bias (IOPcc – Goldmann IOP) was similar in both groups, all eyes at that time being on long term PGA medication, with mean values of 4.1 ± 0.54 mmHg in the control eyes and 4.8 ± 0.54 mmHg in the study eyes. At Visit 2, after a 6 week washout of PGAs, the IOP bias in the tested eye was reduced to 1.6 ± 0.54 mmHg ($p<0.001$), meaning that underestimation of IOP by Goldmann tonometry was significantly less than at Visit 1. The difference in mean IOP bias between the study and control eyes at Visit 2, however, did not

reach statistical significance (p= 0.124). A marginally significant increase of 1.53 ± 0.60 mmHg (p = 0.055) was observed between Visits 2 and 3 in the study eyes, with a final mean IOP bias value of 3.10 ± 0.54 mmHg in the study eyes and 2.8 ± 0.54 mmHg in the control eyes.

We then tried to determine if a low CH was associated with signs of more severe glaucoma progression among our open-angle glaucoma patients treated with PGA. When considering all eyes on PGA at the time of the first Visit, no association was found between VF damage and CH. However, when considering only eyes with more advanced glaucoma, a significant positive correlation was observed between VF MD and CH (B = 0.65; p = 0.003). A lower CH was associated with a more negative visual field MD value and thus greater glaucoma-related damage.

## Conclusions:

Topical prostaglandin analogs reduce CH, CRF, CCT and IOP in glaucomatous eyes. The changes in CH and CCT influence the measurement of IOP, and therefore, these changes should be taken into account when evaluating IOP lowering response to PGA medications. To discern an interaction between IOP and corneal hysteresis, further research should be conducted with intraocular pressure control. It can be achieved by using systemic medications that decrease IOP and do not influence biomechanical properties. It can also be achieved by using Pascal dynamic contour tonometry or a similar tonometer that does not depend on the biomechanical properties of the cornea.

Regarding the interaction between severity of glaucoma damage and corneal hysteresis, it was demonstrated that a lower CH was associated with a more negative visual field MD value.

## Key Words

glaucoma – prostaglandin analogs – corneal hysteresis – central corneal thickness – intraocular pressure – biomechanical properties of the cornea.

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# List of abbreviations

## General

*A:* Anterior

*APG:* les Analogues de Prostaglandines

*CCT:* Central Corneal Thickness

*CH:* Corneal Hysteresis

*CRF:* Corneal Resistance Factor

*CV:* Champ Visuel

*D:* Dioptre

*db:* Decibel

*DM :* Déviation Moyenne

*DM:* Descemet's Membrane

*ECM:* Extracellular Matrix

*ECC :* l'Épaisseur Cornéenne Centrale

*EN:* Endothelium

*FRC :* le Facteur de Résistance Cornéen

*GAO:* Glaucome à l'Angle Ouvert

*HC:* l'Hystérèse Cornéenne

*HRT : Heidelberg Retinal Tomography*

*HTO :* Hypertension Intraoculaire

*IOP :* Intraocular Pressure

*IOPcc:* Corneal-Compensated Intraocular Pressure

*IOPg:* Goldmann-Correlated Pressure

*MD:* Mean Deviation

*MMPs:* Matrix Metalloproteinases

*Na+/K+-ATPase:* Sodium-Potassium Adenosine Triphosphatase

*NSAID'S:* Non-Steroidal Anti-Inflammatory Drug

*OAG:* Open Angle Glaucoma

*OCT:* Optical Coherence Tomography

*OHT:* Ocular Hypertension

*ONH:* Optic Nerve Head

*ORA:* Ocular Response Analyzer

*P:* Posterior

*PGA:* Prostaglandin Analog

*PGAs:* Prostaglandin Analogs

*PIO:* Pression Intraoculaire

*RER:* Rough Endoplasmic Reticulum

*RGC:* Retinal Ganglion Cell

*RNFL:* Retinal Nerve Fiber Layer

*S:* Stroma

*SD:* Standard Deviation

*SE:* Standard Error

*TAG:* la Tonométrie par Applanation de Goldmann

*TEM:* Transmission Electron Microscopy

*TIMPs:* Tissue Inhibitor Metalloproteinases

*VF:* Visual Field

**Units**

*%:* Percent

*°C:* Degree Celsius

*mm:* Millimeter

*mm2:* Millimeter Square

*mg:* Milligram

*mmHg:* Millimeter of mercury

*µg:* Microgram

*µm:* Micrometer

*nm:* Nanometer

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# Introduction

Over the last hundred years, questions concerning the diagnosis and treatment of glaucoma have surfaced. It has been estimated that glaucoma affects sixty-seven million people worldwide, of whom seven million develop blindness. 400,000 Canadians suffer from this disease of the optic nerve and its prevalence is increasing with time.[1,2] Due to the evolution of technology, early diagnosis and appropriate treatment for glaucoma are now possible; however, a “completely cure” treatment for the present optic neuropathy does not currently exist. The search for the most effective treatment for glaucoma as well as for the most informative and predictive risk factors is ongoing.

Previously, intraocular pressure (IOP) was considered the most valuable and informative risk factor and diagnosis for glaucoma. In addition, development of the disease resulted in a significant deviation of IOP from the normal range. In present times, more and more researchers are paying close attention to other potential risk factors, diagnoses and outcomes, including the measurements of corneal thickness and corneal biomechanical properties. Note that these factors have a greater contribution to the development and progression of glaucoma than IOP itself. The explanation behind the importance of the properties and structure of the cornea in glaucoma is presented in the methodology of diagnosis and treatment.

The cornea can be thought of as the window of the eye. It can be used in order to observe events occurring within the eyeball, to determine the intraocular pressure and to administer medication to the eye. During the measurement of intraocular

pressure, the cornea is flattened or applanated. The properties and structure of the cornea have an impact on the applanation event as a thicker and stiffer cornea requires a greater applied force to achieve applanation compared to a thinner and weaker cornea.[5] Thus, corneal properties and structure influence IOP measurements. The administration of eye drops, such as Prostaglandin Analogs (PGA), directly onto the cornea can also affect its structure and properties.[6-9] On the other hand, the cornea can influence drop action and effectiveness.[10-22]
This study was conducted to better understand the association between the corneal biomechanical properties and the treatment, diagnosis and outcome of glaucoma.

## Research Objectives and Study Design

## Objectives:

The primary objective is to determine whether the use of topical prostaglandin analog medication affects biomechanical properties of cornea. The conclusion will be based on the integrated analysis of the results obtained from the Reichert Ocular Response Analyzer (ORA), Goldmann tonometer and ultrasound pachymeter.

The secondary aim of this study is to determine the associations between the corneal biomechanical properties, central corneal thickness (CCT) and intraocular pressure (IOP) in patients undergoing topical PGA medication treatment.

## Hypothesis:

PGA drops influence the central corneal thickness and corneal biomechanical properties including corneal hysteresis, elasticity and resistance.

## Study Design:

A. Patients

Thirty five patients (seventy eyes) between the ages of 50 and 85 years with open angle glaucoma (OAG) treated with topical PGA medications were recruited from the glaucoma clinics of the Montreal Glaucoma Institute. One of the patient's eyes served as the experimental eye, while the contralateral eye served as the control for statistical analyses.

B. Inclusion Criteria and Patient Definitions

Recruited patients with OAG had gonioscopically open angles and a manifest refraction between -6.00 D and +4.25 D. Early glaucoma is arbitrarily defined as a mean defect of -2.00 to -6.00 db and moderate glaucoma as a mean defect of -6.10 to -12.0 db.

C. Exclusion Criteria

Excluded individuals consisted of patients with any other corneal eye disease, such as Fuch's endothelial dystrophy or keratoconus, or any past history of corneal trauma or surgery, including refractive surgery that may affect hysteresis measurements. Due to the fact that systemic medications may have an effect on the cornea and, therefore, alter the results of the study, patients using systemic prostaglandin pills, non-steroidal anti-inflammatory drugs or undergoing hormone replacement therapy were excluded. Contact lens wearers and patients with

uncontrolled glaucoma or far advanced visual field (VF) damage were also excluded.

D. Study Procedures

Following informed consent, patients undergoing topical PGA treatment in both eyes were asked to discontinue the PGA in their best eye (less damage on VF – better MD or lower max IOP if MD equal) and to continue the administration of PGA in the contralateral eye. Corneal measurements were taken before PGA cessation (Time 1) and repeated 6 weeks after cessation (Time 2). Patients then restarted the application of PGA to the experimental eye and all measurements were repeated once more after an additional 6 weeks (Time 3). After starting or changing glaucoma treatment the patient should be seen approximately 4-6 weeks later to assess efficacy of the drop.[4] For this reason it was decided to use 6 weeks interval. All measurements were performed at the Montreal Glaucoma Institute by the same trained technician, with the same equipment and at the same time of day.

E. Data Collection

Goldmann applanation tonometry (Haag-Streit AG, Koeniz, Switzerland) is the gold standard for measuring IOP in glaucoma patients. IOP was measured with the same calibrated tonometer during the study.

Central Corneal Thickness (CCT) was measured by ultrasound pachymetry (DGH Technology, INC, Exton, PA, USA) and the average of three measurements was recorded.

The Ocular Response Analyzer (ORA) (Reichert, INC, Depew, NY, USA) utilizes a dynamic bi-directional applanation process to measure the biomechanical properties of cornea and the IOP of the eye. Using this device, biomechanical properties, including Corneal Hysteresis (CH), Corneal Resistance Factor (CRF), Corneal-Compensated Intraocular Pressure (IOPcc) and Goldmann-Correlated Pressure (IOPg), can be measured. The ORA was used to classify various corneal conditions based on biomechanical tissue properties, rather than geometrical measurements.

The ORA utilizes a rapid air pulse to apply force to the cornea. An advanced electro-optical system monitors corneal deformation. A precisely-metered collimated-air-pulse causes the cornea to move inwards into slight concavity. Milliseconds after applanation, the air pump shuts off and the pressure gradually declines. As the pressure decreases, the cornea first passes through an applanated state before resuming its original curved structure. The applanation detection system monitors the cornea throughout the entire process, and two independent pressure values are derived from the INWARD and OUTWARD applanation events. The difference between these two pressure values is Corneal Hysteresis (CH). The CH is a measure of viscous damping in the cornea. The ability to measure this effect is the key to understanding the biomechanical properties of the cornea and their influence on the IOP measurement process. The normal value of CH is around 11.00 – 12.00 mmHg. [23]

The ORA was used for the measurements of CH (like a value of corneal biomechanical properties in general) and to verify the IOP. Four ORA

measurements were taken per eye and the mean value was recorded. The same ORA was used for all of the patients during the study.

The CH measurement also provides a basis for two additional new parameters: IOPcc and CRF. IOPcc is an IOP measurement that utilizes the new information provided by the CH measurement to provide an IOP value that is less affected by corneal properties than other methods of tonometry, such as GAT. CRF is measurement of the cumulative effects of both the viscous and elastic resistance encountered by air jet while deforming the cornea. The normal value of CRF is around 10.00 – 11.00 mmHg.[23]

All of the necessary equipment needed for this research was calibrated before the start and during the project to avoid imprecise measurements.

F. Data Management

Baseline and follow-up data was entered into an Excel spreadsheet. Statistical analyses were performed using the appropriate tests.

G. Statistical Analysis

The hypothesis of no effect regarding the discontinuation of PGA on the biomechanical properties was examined by a linear mixed-effect model using the nlme package in R. Random-effects was defined on two levels: the patient (level-1) and the eye within each patient (level-2). Those random-effects were added to the model to account for the intra-individual variance due to the repeated-measure design. Age was also included in the model as a covariate. Contrasts between the eyes and times were estimated using adjusted p-values to control for familywise error rate using multcomp package in R.[24]

H. Ethical Considerations

The approval was obtained by the ethics committee of Maisonneuve-Rosemont Hospital. Prospective candidates were thoroughly informed of the protocol and had the opportunity to have any questions answered before being asked for informed consent. Each patient was given a code and all data was masked to protect patient confidentiality.

Ocular exams, IOP, pachymetry and ORA are part of standard care for human testing and pose minimal risk to subjects. These studies will contribute towards a better understanding of Glaucoma and, eventually, to better management.

This study was conducted in accordance with research protocol and recommendation of the ethics commission. All ethical issues connected with this study were resolved in accordance with applicable law and ethical principles.

# *Chapter I:* The Normal Human Cornea

## 1.1 Anatomy of the Human Cornea

The cornea is the anterior transparent connective tissue of the eye. It is responsible for protecting the eye from injury and infection. It also provides the majority (two thirds) of the total refractive power of the eye.[25]

The cornea is comprised of five layers (see Figure 1): the outermost non-keratinised stratified epithelium, Bowman's layer, a highly ordered keratocyte-populated collagenous stroma, Descemet's membrane and the inner endothelium (a cellular monolayer).

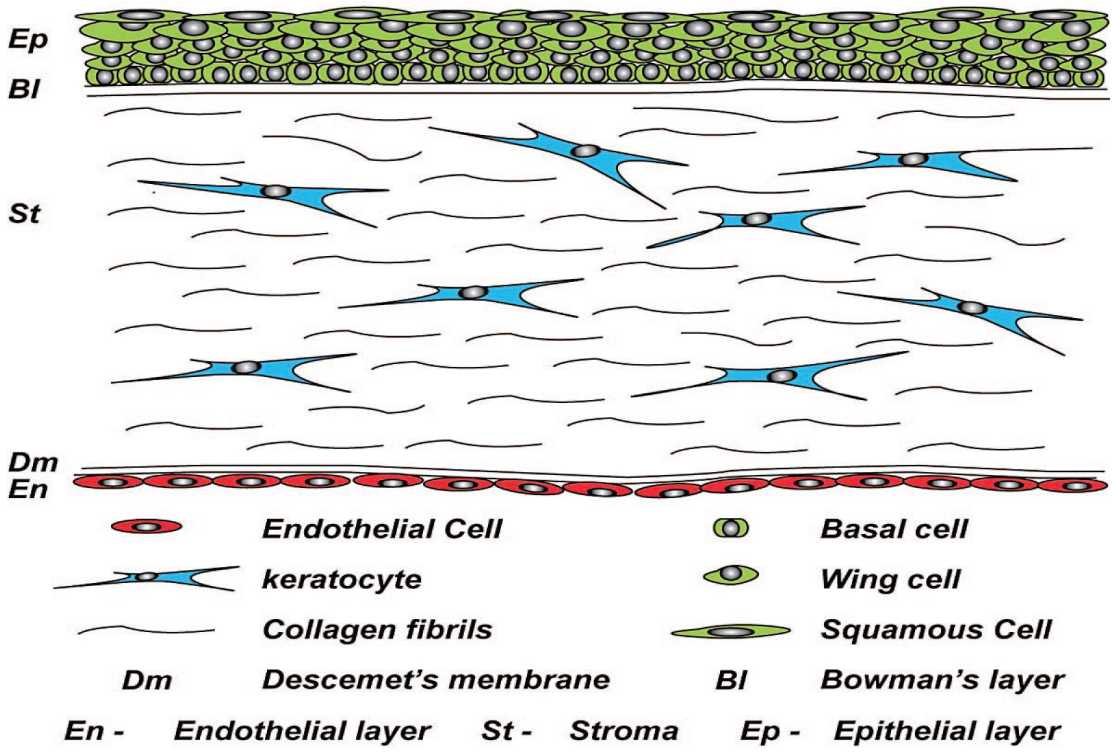


**Figure 1: The human cornea in cross-section (from: http://www.stembook.org).**

In emmetropic adults, the average white-to-white diameter of the cornea is 11.5 to 12.3 mm. The central corneal thickness ranges from 528 to 588 µm and gradually increases towards the periphery.[26]

### *1.1.1 Epithelium*

The corneal epithelium forms the first barrier separating the eye from the external environment. It is a stratified non-keratinized squamous epithelium containing 4 to 6 cell layers (40 to 50 μm in thickness). These layers are divided from posterior to anterior into: basal, wing and superficial cells. Corneal epithelial lifespan is approximately 1 week. During that short period, the cells go through systematic involution, apoptosis and desquamation. This process leads to a complete renewal of the epithelium every week.[27] The differentiated squamous cells have surface microvilli and occupy the outer 1–3 cell layers of the epithelium (Figure 2). The function of the microvilli is to increase cell surface area, forming a close association with the tear film. Highly resistant tight junctions between neighbouring cells form a protective barrier.[10]

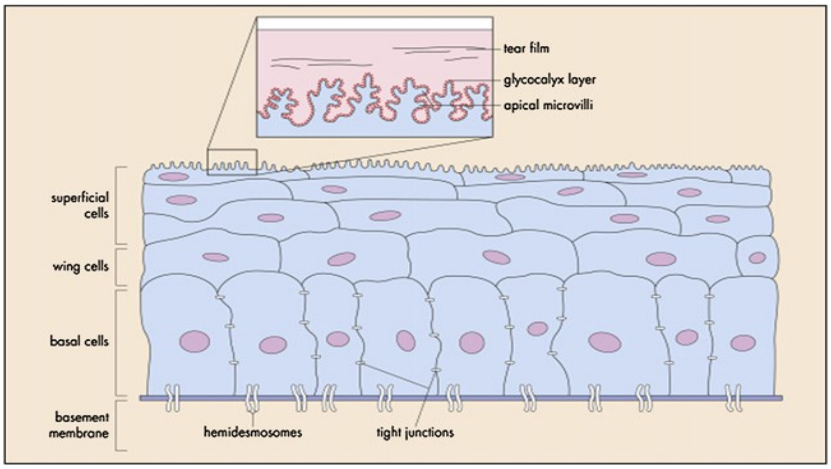


**Figure 2: Corneal epithelial layers**[11]

The inner basal cells consist of a single layer of columnar cells (~20 μm tall). Their functions include the generation of new suprabasal cells, the secretion of

matrix factors and the regulation of the organisation of hemidesmonsomes and focal complexes to maintain attachment to the underlying basement membrane. They also secrete their basement membrane (0.05 μm in thickness) containing collagen type IV and laminin.[11] These functions are believed to be important in mediating cell migration in response to epithelial injury.[12]

### *1.1.2 Bowman's Membrane*

Bowman's membrane (10-15 μm in thickness) lies posterior to the basement membrane of the epithelium. It is not a true membrane as it is an acellular condensation of collagen fibers and proteoglycans of the anterior portion of the stroma. It does not exist in many mammals. In cases of injury, this membrane does not regenerate and forms a scar.[11]

### *1.1.3 Stroma*

The stroma is a mesenchymal tissue derived from the neural crest. It constitutes 85% to 90% of corneal thickness. The central stroma is thinner than the peripheral one, and the collagen fibers change their direction and run circumferentially when they arrive near the limbus.[13] Stromal collagen fibers consist mainly of collagen types I and V to achieve their uniform diameter. Collagen types VI and XII bind other types of collagen fibers and help to maintain the regularity of the stromal structure. Keratan sulfate and chondroitin sulfate/dermatan sulfate, which make up the ground substance of the stroma, play a role in regulating the hydration state and the uniform structure of the stroma.

The transparency of the stroma is unique among other collagenous structures. The parallel arrangement of lamellae formed from heterodimeric complexes of type I and type V collagen fibers maintain transparency.[14] The keratocytes (fibroblasts) located between the lamellae[15] link to one another via dendritic processes[16], producing crystalline proteins that also help maintain corneal transparency.[17]

The corneal endothelium plays a critical role in maintaining the relatively dehydrated state of the stroma, thus preserving the precise organisation of the stromal collagen fibers. These collagen fibres are held in a uniform spacing pattern by proteoglycans.

The posterior lamellae are more arranged and less rigid than the anterior ones. This biomechanical difference is translated clinically in a stromal edema that is more marked posteriorly, pushing on the Descemet's membrane and creating Descemet folds.[11]

### *1.1.4 Descemet's Membrane*

The Descemet's membrane (DM) rests on the innermost surface of the cornea. It acts as a basement membrane for the inner endothelial cell monolayer. The normal DM is composed of two layers (Figure 3):

- Anterior banded layer: It is present in the eye of the fetus at five months of gestation. Its thickness is approximately 3 μm and it remains the same at all ages.[18]

- Posterior nonbanded layer: It is secreted onto the posterior surface of the banded fetal portion after birth by the endothelial cells. Its thickness increases

significantly with age, averaging ~2 μm at 10 years of age and ~10 μm at 80 years of age.[19]

In healthy adults, the DM is composed of collagen type IV (dominant), VIII and XII, laminin, perlecan, nidogen-1, nidogen-2, netrin-4, vitronectin and fibronectin.[18,20] These cells transport nutrients from the aqueous humour to the stroma. In addition, they pump out excess water and prevent corneal edema (swelling) by maintaining optimal hydration.

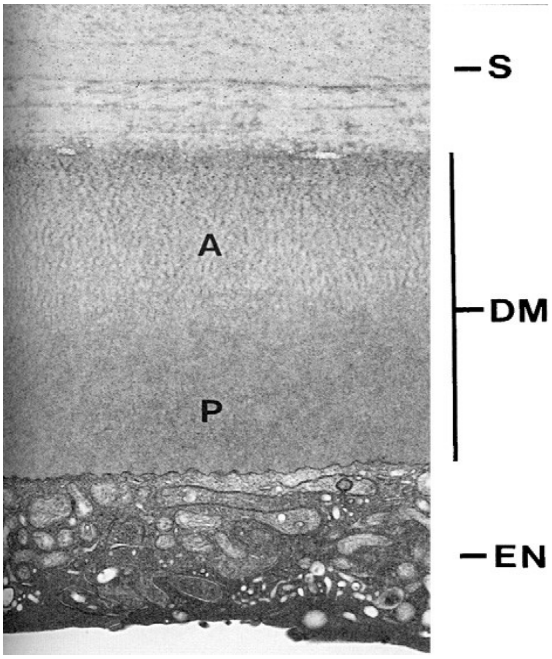


**Figure 3: Transmission electron microscopy (TEM) of Descemet's membrane (DM) showing posterior stroma (S), anterior (A) and posterior (P) layers of normal DM, and endothelium (EN)[11]**

### *1.1.5 Endothelium*

Corneal specialists consider the endothelium as the soul of the cornea.

At approximately the sixth week of gestation, corneal endothelial cells originating from the neural crest (neuroectoderm) migrate centrally from the rim of the optic

cup to form a monolayer of cubical cells.[21] Over time, these cells flatten and start to secrete their basal membrane (DM).

The DM fuses peripherally with the trabecular beams where the Schwalbe line defines the ending of DM and the beginning of the trabecular meshwork.[11]

It appears as a uniform honeycomb-like mosaic with four to nine sides, about 20μm in diameter and 250 μm² in cell area (Figure 4).

The lateral membranes enclose a high density of $Na^+$/ $K^+$-ATPase pump sites. An individual endothelial cell contains a large number of oblong nuclei, numerous mitochondria, a rough endoplasmic reticulum (RER), and a prominent Golgi apparatus (indicating elevated levels of protein synthesis including enzymes, structural proteins and extracellular matrix (ECM) proteins) (Figure 4).[21, 11]

The central endothelial cell density decreases at an average rate of 0.6% per year in normal corneas.[22] It was also found that there is no correlation between corneal thickness and endothelial cell density, cell area, coefficient of variation or cell shape.[11]

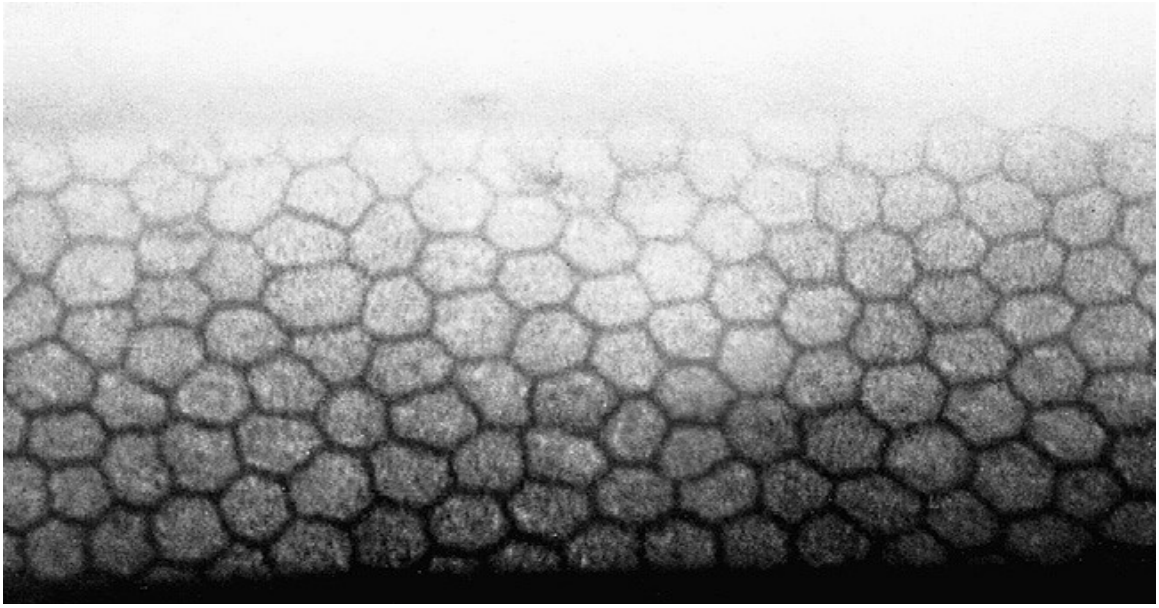

**Figure 4: Specular microscopy photo of the normal corneal endothelium. Note the regular hexagonal pattern[11]**

## 1.2 Physiology of the Human Cornea

### *1.2.1 Biomechanical Properties of the Cornea*

The human cornea is considered “the window” of the eye, enabling a professional to see the events occurring within the eye. The transparency of the cornea is one of several corneal properties, which vary according to the different corneal functions.

The cornea consists primarily of collagen, a viscous and elastic material. The viscoelasticity provides a measure of corneal biomechanics and protects against mechanical and biological damages. The visco-elastic properties of the cornea reflect its ability to absorb the energy of the external force and bend during compression.[5, 28]

In clinics, the corneal viscoelasticity is used to verify intraocular pressure. During the measurement of intraocular pressure, the cornea is deformed. This deformation is determined by the interaction between the external force and the corneal visco-elastic properties. A greater deformation occurs in response to lower intraocular pressure; however it is also associated with a “weaker” and “softer” cornea. A smaller corneal deformation not only indicates a higher intraocular pressure but also that the cornea is “stiff” and “hard”.

Corneal hysteresis (CH) is a corneal biomechanical property and it can be measured using the ocular response analyzer. [29]

# Chapter II: Glaucoma

## 2.1 Anatomy and Definition of Glaucoma

Glaucoma is the leading cause of irreversible blindness throughout the world[1], affecting an estimated 67 million individuals of whom approximately 10 percent are bilaterally blind. It is also the second leading cause of blindness and visual handicap in Canada, affecting 1% of the Canadian population. [2] The prevalence of glaucoma rises dramatically with increasing age. In fact, it is known to rise exponentially after the age of 40.[30]

Glaucoma is a chronic and generally bilateral but often asymmetrical eye disease. It is characterized by damage to the optic nerve that is usually caused by excessively high intraocular pressure (IOP).

### *2.1.1 Anatomy of the Optic Nerve Head*

The optic nerve head (ONH), also known clinically as the optic disc or papilla, forms the exit point for retinal ganglion cell (RGC) axons through the scleral canal. It consists mainly of nerve fibers (1.2-1.5 million retinal ganglion cell axons), glial cells, extracellular matrix supporting tissue and vascular elements.[31-33] The ONH stands out from the neighboring peripapillary tissue by a scleral rim of connective tissue known as the border tissue of Eischnig.[34]

The ONH is divided into four anatomical areas from front to back[35-38]:

A. Surface nerve fiber layer: a continuous layer of retinal nerve fibers. It consists of non-myelinated axons of RGCs in transition from the surface of the retina to the neural component of the optic nerve.

B. Prelaminar area: an area between the surface layer of nerve fibers and the lamina cribrosa at the level of the choroid and the outer retina. It consists of

nerve fibers arranged in bundles surrounded by glial tissue septa and astrocytes.

C. Lamina cribrosa region: adjacent to the sclera and penetrates it. The lamina cribrosa region also provides substantive support to the optic nerve at the exit of the eye.

D. Retrolaminar region: this region is located behind the lamina cribrosa. It is marked by the beginning of myelination of axons and surrounded by leptomeninges of the central nervous system.

The differences between these four regions reflect the conditions in which axons pass through the ONH. These differences include myelination of axons posterior to lamina cribrosa, sources of blood supply, changes intraocular pressure and cerebrospinal fluid pressure.

### *2.1.2 Anatomy of the Anterior Segment of Eyeball*

The anterior segment consists of the cornea, the anterior and posterior chambers, iris, lens and others (Figure 5).

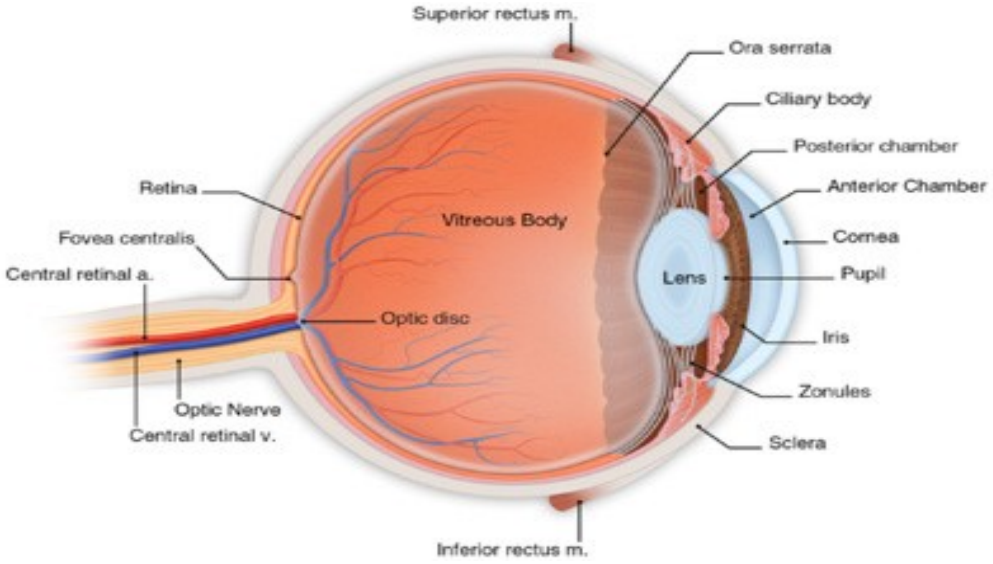


**Figure 5. Anatomy of the eye**
**(from:** www.marineyes.com)

The angle of the anterior chamber (where the iris meets the cornea) plays a critical role in the pathophysiology of glaucoma. It consists of the ciliary body, scleral spur, trabeculae (system funnels for filtration of liquid) and Schwalbe line (Figure 6).

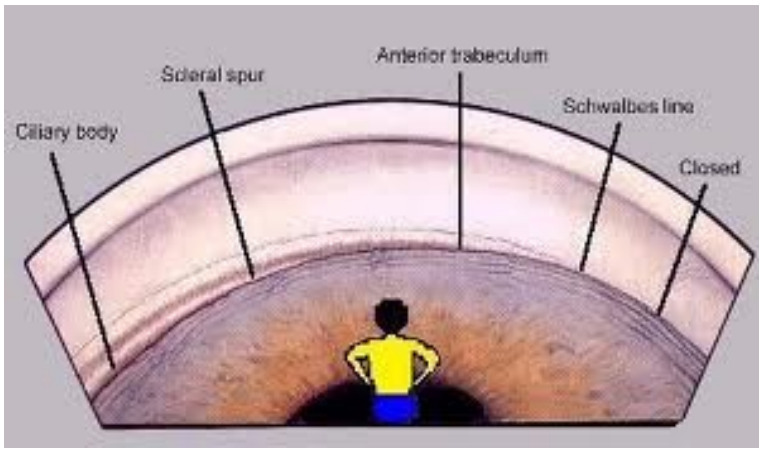


**Figure 6. Anatomy of anterior chamber**
**(from:** www.academy.org.uk**)**

The iris is connected to the sclera via a mesh-like fibrillar structure called the *trabecular meshwork or trabeculae.* The ciliary body produces a fluid that provides nutrients to the lens and cornea (two structures in anterior segment of the eye that do not have a blood supply). This fluid also flows around the lens, through the pupil of the iris, into the anterior chamber and then exits through the trabecular meshwork. Behind this meshwork lies a circumferential tubular structure called the canal of Schlemm. This canal is connected to the venous outflow of the eye through a series of interconnecting tubules called the collector channels (Figure 7). [39, 3]

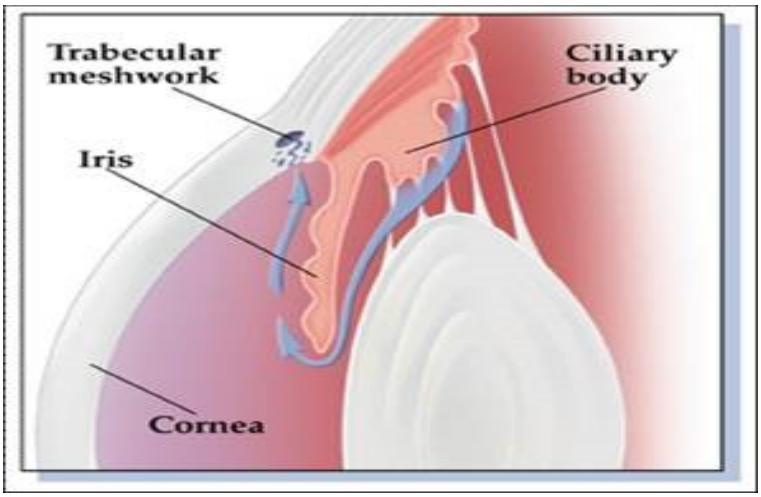


**Figure 7. Anatomy of trabecular meshwork and intra-ocular fluid circulation (from:** www.pharmacologydopt.blogspot.com**)**

## 2.2 Pathophysiology of Glaucoma

### *2.2.1 Types of Glaucoma*

Due to distinct anatomical and physiological features, specialists can distinguish the following types of glaucoma: open-angle glaucoma, closed-angle glaucoma, pigment dispersion syndrome, exfoliation syndrome, normal tension glaucoma, secondary glaucoma and others.

Primary open angle glaucoma is by far the most common form of the disease in North America, affecting Caucasians and persons of African ancestry. Its incidence increases with age. Primary open angle glaucoma is defined as a chronic, generally bilateral, but also asymmetrical disease characterized by all of the following[40]:

1. Evidence of glaucomatous optic nerve damage.

2. Adult onset.

3. Open normal-appearing anterior chamber angles.

4. Absence of known other causes of open angle glaucoma.

### *2.2.2 Risk Factors for Glaucoma*

Previously, the measurement of intraocular pressure (IOP) was considered the most valuable and informative risk factor and diagnosis for glaucoma. In addition, development of the disease resulted in a significant deviation of IOP from the normal range. In present times, more and more researchers have begun to pay close attention to other potential risk factors, diagnoses and outcomes, including the measurements of corneal thickness and corneal biomechanical properties. Note that these factors have a greater contribution to the development and progression of glaucoma than IOP itself.[3]

## 2.3 Methods of Diagnosis of Glaucoma

Diagnosing a patient with glaucoma is a difficult task for physicians. Even though additional tools for diagnosing glaucoma were invented, such as the Heidelberg Retinal Tomograph (HRT) and Optical Coherence Tomography (OCT), the primary methods of diagnosis are still IOP measurement, ophthalmoscopy of the optic disc and visual field testing.

Due to the discovery of additional risk factors, the measurements of central corneal thickness and corneal biomechanical properties have been integrated into routine practice.

Let's look at these methods of diagnosis in more detail.

The Humphrey Visual Field test is used to define visual field defects, which are characteristic of glaucoma. This is a useful tool for diagnosis and glaucoma follow-up.

Ophthalmoscopy of the optic disc and retina, performed by ophthalmologist, is used to verify optic nerve cupping and changes in retinal structures.

Heidelberg Retinal Tomograph (HRT) is a confocal laser scanning system used for acquisition and analysis of three-dimensional images of the posterior segment of the eye. HRT measures linear Cup/Disk ratio, Rim area and RNFL thickness. It can be used for diagnosis as well as for glaucoma follow-up.

Optical Coherence Tomography (OCT) is a non-invasive imaging test that uses light waves to take cross-section pictures of the retina and optic nerve disk. OCT can measure Cup/Disk ratio, Rim area, RNFL thickness and ganglion cells analysis. It can be used for diagnosis as well as for glaucoma follow-up.

Goldmann applanation tonometry is the gold standard for measuring IOP in glaucoma patients. It can be used for the diagnosis of glaucoma and pressure control. A special disinfected prism is mounted on the head of the tonometer and then placed against the cornea. The examiner then uses a cobalt blue filter to view two green semi circles. The force applied to the tonometer head, which flattens the cornea, is used to measure IOP. Like all non-invasive methods, it is inherently imprecise.

### *2.3.1 Measurements of Biomechanical Properties of the Cornea*

Central Corneal Thickness (CCT) can be measured by ultrasound pachymetry. It has recently been identified as a risk factor for glaucoma development and is considered during the correction of IOP measurements and glaucoma follow-up.

According to measurements of CCT and tonometery presented in Figure 8, the patient's true pressure can be calculated. Thinner corneas are associated with higher IOP values and vice versa.

| Corneal Thickness (µm) | Correction Value |
|---|---|
| 405 | 7 |
| 425 | 6 |
| 445 | 5 |
| 465 | 4 |
| 485 | 3 |
| 505 | 2 |
| 525 | 1 |
| 545 | 0 |
| 565 | -1 |
| 585 | -2 |
| 605 | -3 |
| 625 | -4 |
| 645 | -5 |
| 665 | -6 |
| 685 | -7 |
| 705 | -8 |
| Correction values according to corneal thickness of 545 µm | |

**Figure 8. Correction values of IOP according to corneal thickness (from:** www.emedicine.medscape.com)

The biomechanical properties of the cornea are measured using the ocular response analyzer (ORA). This device measures the corneal hysteresis, a newly discovered risk factor for glaucoma development that is also analyzed during glaucoma follow-up.

The ORA utilizes a dynamic bi-directional applanation process to measure the biomechanical properties of cornea and the IOP. A rapid air pulse applies force to the cornea and an advanced electro-optical system monitors corneal

deformation. A precisely-metered collimated-air-pulse pushes the cornea inwards, past a flat state and into a slight concavity. Milliseconds after applanation, the air pump shuts off and the pressure gradually declines. As the pressure decreases, the cornea first passes through an applanated state before resuming its original curved structure. The applanation detection system monitors the corneal movement throughout the entire process. Two separate pressure values are derived from the INWARD and OUTWARD applanation events. The difference between these two pressure values is termed Corneal Hysteresis (CH). The CH is a measure of viscous damping in the cornea. The ability to measure this effect is the key to understanding the biomechanical properties of the cornea and their influence on the IOP measurement process. The normal value of CH is around 11.00 – 12.00 mmHg. [23]

The ORA can be used to classify various corneal conditions based on biomechanical tissue properties rather than geometrical measurements. The ORA enables the measurements of corneal biomechanical properties (Figure 9) such us Corneal Hysteresis (CH), Corneal Resistance Factor (CRF), Corneal-Compensated Intraocular Pressure (IOPcc) and Goldmann-Correlated Pressure (IOPg).[29]

The CH measurement also provides a basis for two additional new parameters: IOPcc and CRF. IOPcc is an IOP measurement that utilizes the new information provided by the CH measurement to provide an IOP value that is less affected by corneal properties than other methods of tonometry, such as GAT. CRF is measurement of the cumulative effects of both the viscous and elastic resistance

encountered by air jet while deforming the cornea. The normal value of CRF is around 10.00 – 11.00 mmHg.[23]

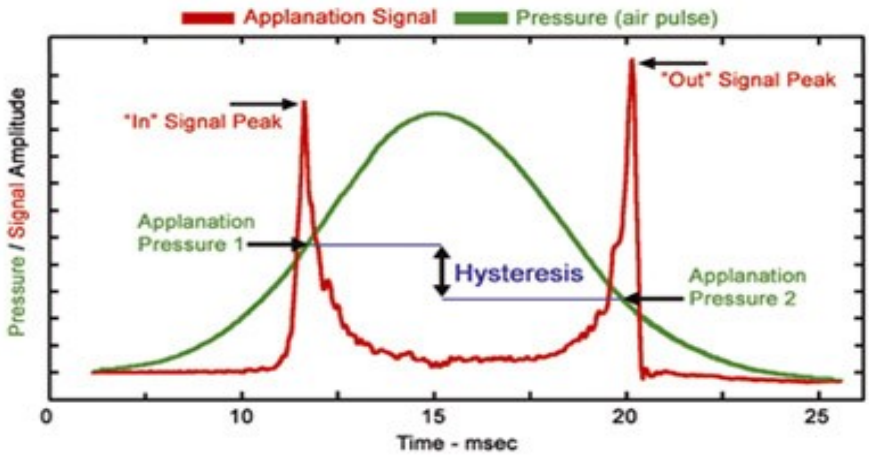


**Figure 9. The ocular response analyzer measurement curve (from:** www.reichert.com**)**

The biomechanical properties of the cornea play an extraordinary role in diagnosis of glaucoma as well as in course of the disease and prognosis. The individual with low CCT and CH has a greater risk of developing glaucoma. Low CCT and CH are also associated with a more rapid progression of the disease.

## 2.4 Treatment of Glaucoma

The treatments for glaucoma can be classified as medical, laser or surgical. Laser and surgical treatments are usually used for patients with advanced stages of glaucoma as well as for those that do not respond to medication. Medication is administered in the form of drops and different types of eye drops have different functions. There are those that reduce the production of intra-ocular fluid (B-blockers and Brinzolamide), drops that improve anterior outflow tracts (Pilocarpine) and drops that improve anterior and posterior outflow paths

(prostaglandin analogs (PGA)). The search for the most effective treatment for glaucoma, defined as the best method to stabilize the circulation of intraocular fluid, is ongoing.

### *2.4.1 Topical PGA Medications, Mechanism of Actions*

Topical PGAs are currently the first choice of intraocular pressure reducing medication. Among them are several distinguished groups of drugs such as Bimatoprost, Latanoprost, Travoprost and Tafluprost. The mechanism of action of these drops is similar; however, there are some differences.

Prostaglandin analogs reduce pressure inside the eye (IOP) by increasing the uveoscleral outflow, thus increasing the removal of the aqueous humor from the eye. The exact mechanism for this is unknown. Meanwhile, one well-studied mechanism by which PGA increases the uveoscleral outflow involves the regulation of matrix metalloproteinases and remodeling of extracellular matrix. Other proposed mechanisms include widening of the connective tissue-filled spaces and changes in the shape of cells. The final steps of all these mechanisms alter the permeability of the tissues of the outflow pathways leading to changes in outflow resistance and/or outflow rates. Prostaglandin receptors and their associated mRNAs have been located in the trabecular meshwork, ciliary muscle, and sclera, providing evidence that endogenous prostaglandins have a functional role in aqueous humor drainage.[6] In addition, it was recently found that the same PGA receptors are also found in the cornea as well.[41-48,7-9] This can explain the actions of PGA on corneal properties (Figure 10).

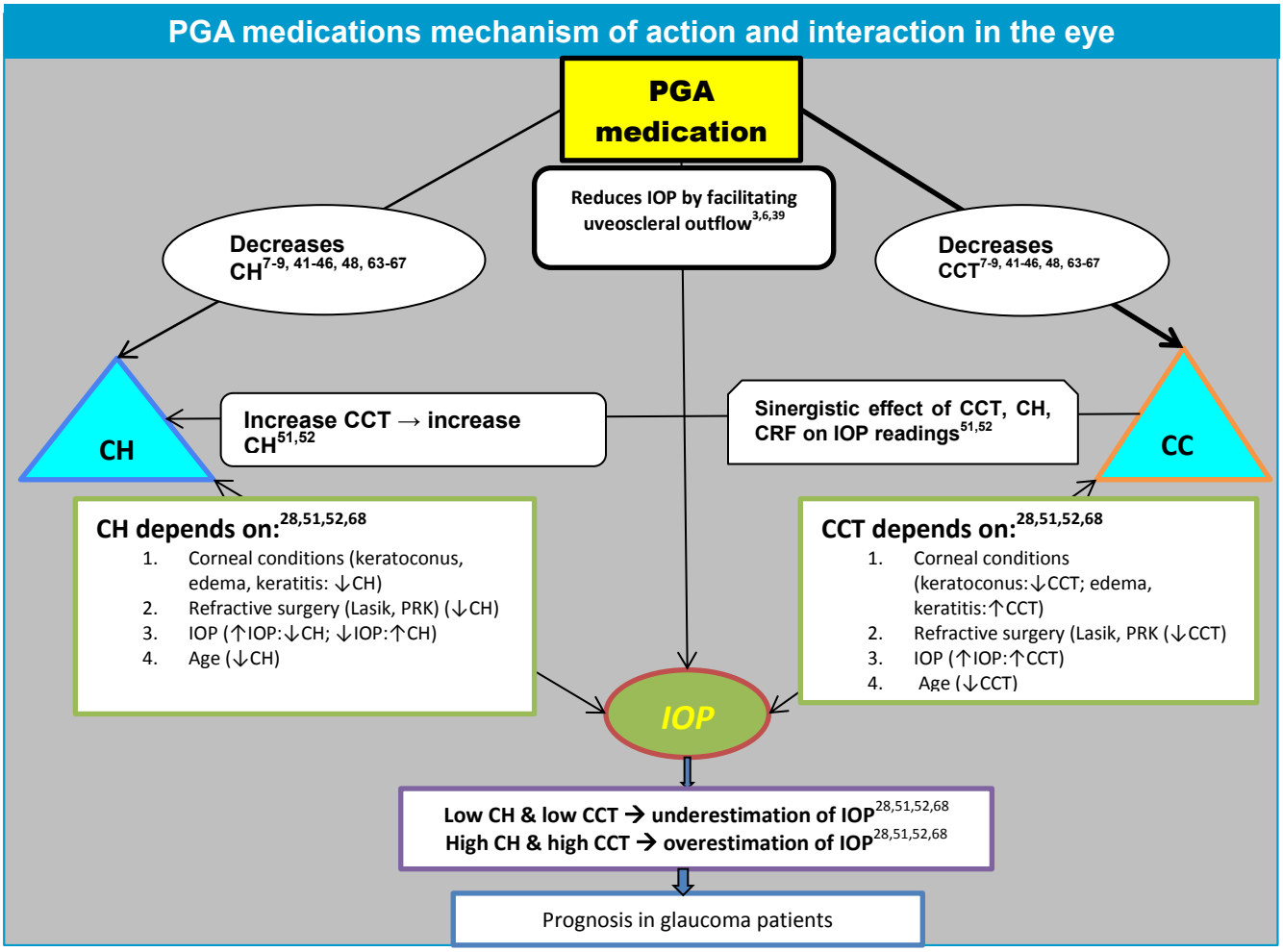


**Figure 10: The action and interaction of PGA medication in the eye**

# *Chapter III:* The Impact of Topical Prostaglandin Analogs on the Biomechanical Properties of the Cornea in Patients with Open Angle Glaucoma: *Article*

# The Impact of Topical Prostaglandin Analogs on the Biomechanical Properties of the Cornea in Patients with Open Angle Glaucoma

(Manuscript prepared for submission in Ophthalmology, September 2014)

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## 3.3 Abstract

**Purpose:** To determine the influence of prostaglandin analog (PGA) medication on corneal biomechanical properties in patients undergoing PGA treatment for open-angle glaucoma.

**Participants:** Seventy eyes of 35 patients, aged 50 to 85 years, with open-angle glaucoma and undergoing topical PGA treatment were recruited for the study. One of the patient's eyes served as the experimental eye, while the contralateral eye served as the control.

**Methods:** From a treated baseline (Visit 1), patients discontinued the application of PGA in their "best" eye for a period of 6 weeks (Visit 2). Intraocular pressure (IOP), corneal hysteresis (CH) and central corneal thickness (CCT) were measured and recorded. Following Visit 2, patients resumed the application of PGA to the experimental eye for a final 6-week period and the measurements were repeated (Visit 3).

**Main Outcome Measures:** IOP, CH and CCT were measured using a Goldmann applanation tonometer, Reichert Ocular Response Analyzer and ultrasound pachymetry.

**Results:** Mean CH (±Standard Error) decreased from 10.35 ± 0.28 mmHg to 8.98 ± 0.28 mmHg following the PGA treatment in the experimental eye. CCT, corneal resistance factor (CRF) and Goldmann IOP increased by 10.09 ± 0.94 μm ($p < 0.001$), 1.49 ± 0.21 mmHg ($p < 0.001$) and 3.0 ± 0.49 mmHg ($p < 0.001$) respectively, after PGA cessation. The data suggests that patients undergoing PGA therapy are subject to greater underestimation of IOP as measured by the

Goldmann applanation tonometer.

**Conclusion:** Topical PGA reduces CH, CRF, CCT and IOP in patients suffering from open-angle glaucoma. Deviations of CH and CCT values from the normal range contribute to the false IOP readings made by the Goldmann applanation tonometer and should be considered when measuring IOP to monitor the response to treatment.

## 3.4 Introduction

Glaucoma affects approximately 67 million people worldwide, of whom seven million develop blindness. Despite significant progress in pharmacotherapy, laser technology, and surgical techniques, the treatment of glaucoma remains a challenge.[1] Early diagnosis and accurate assessment of glaucoma progression and response to treatment are essential in order to adjust treatment and minimize the risk of permanent blindness.

While intraocular pressure (IOP) has long been considered the most valuable indicator of glaucoma progression, closer attention is now paid to other potential risk factors susceptible to influence diagnosis and outcome of glaucoma such as corneal thickness and corneal biomechanical properties.[3]

Corneal Hysteresis (CH) and Corneal Resistance Factor (CRF) are classified as corneal biomechanical properties. CH represents the viscous damping occurring within the cornea in response to an applied force. Thus, a low CH reflects a lack of energy absorption. CRF, on the other hand, is a measure of corneal rigidity dependent on the elastic and viscous materials of the cornea. A reduction in CRF signifies a lack of resistance to deformation.[49]

The cornea is primarily composed of collagen, a documented viscoelastic material. During Goldmann applanation tonometry (GAT), the cornea is flattened or applanated. Applanation is affected by the biomechanical properties of the cornea as a thicker and stiffer cornea requires a greater applied force to achieve applanation compared to a thinner and weaker cornea. Corneal hysteresis (CH) represents the viscoelasticity of the cornea. This biomechanical property is existent due to the collagen content and composition of the corneal extracellular matrix.[50]

The objective of the study was to determine whether the use of topical PGA medication affects CH and CRF in eyes with open-angle glaucoma.

## 3.5 Methods

### Patients

Thirty five patients (seventy eyes) with bilateral open angle glaucoma on monotherapy with topical PGA medication in both eyes were recruited from the Montreal Glaucoma Institute, Montreal, QC, Canada. Exclusion criteria included any corneal disease (such as Fuchs' endothelial dystrophy or keratoconus) or a past history of corneal trauma or surgery (including refractive surgery) that may affect hysteresis measurements. Contact lens wearers and patients with uncontrolled glaucoma or advanced visual field (VF) damage (mean defect < -12.0 db) were excluded. Patients taking systemic prostaglandin medication, non-steroidal anti-inflammatory drugs or undergoing hormone replacement therapy were also excluded from this study. Informed consent was acquired from all participants. The research protocol of this study adhered to the tenets of the

Declaration of Helsinki. It was approved by the Maisonneuve-Rosemont Hospital Ethics Committee (Montreal, QC, Canada) and all patients signed a research consent form.

**Study Procedures**

Consenting patients undergoing topical PGA treatment in both eyes were asked to discontinue the PGA in their best eye and to continue the administration of PGA in the contralateral eye. All measurements were taken before PGA cessation (Visit 1) and repeated 6 weeks after cessation (Visit 2). Patients then restarted the application of PGA to the experimental eye and all measurements were repeated once more after an additional 6 weeks (Visit 3). After starting or changing glaucoma treatment the patient should be seen approximately 4-6 weeks later to assess efficacy of the drop.[4] For this reason it was decided to use 6 weeks interval.

The best eye was selected based on less glaucoma damage. It was defined by the results of the Humphrey Visual Field (HFA, Carl Zeiss Meditec, Inc., Dublin, CA), Heidelberg Retinal Tomograph (HRT II, Heidelberg Engineering GmbH, Heidelberg, Germany) and Optical Coherence Tomography (CIRRUS HD-OCT, Carl Zeiss Meditec, Inc., Dublin, CA). The Humphrey Visual Field defines early glaucoma as a mean defect (MD) of -2.00 to -6.00 db, moderate glaucoma as a MD of -6.10 to -12.0 db and advanced as a MD less than -12.0 db. The Optical Coherence Tomography enables the identification of the better linear Cup/Disk ratio, Rim area, RNFL thickness and ganglion cells analysis between both of the

patient's eyes. The lesser of the maximum IOP values recorded in each of the patient's eyes also contributed to the selection of the best eye.

**Instruments**

The Ocular Response Analyzer (ORA) (Reichert, INC, Depew, NY) was used to assess the biomechanical properties of the cornea. This instrument utilizes a dynamic bi-directional applanation process to measure the biomechanical properties of the cornea and estimate IOP. A rapid air pulse applies force to the cornea and an advanced electro-optical system monitors corneal deformation. A precisely-metered collimated-air-pulse pushes the cornea inwards, past a flat state and into a slight concavity. Milliseconds after applanation, the air pump shuts off and the pressure gradually declines. As the pressure decreases, the cornea first passes through an applanated state before resuming its original curved structure. The applanation detection system monitors the corneal movement throughout the entire process. Two separate pressure values are derived from the INWARD and OUTWARD applanation events. The difference between these two pressure values is termed CH.[29] This biomechanical property reflects corneal absorption and dissipation of the energy from an applied force.[29] Derived from the measurement of CH is the corneal resistance factor (CRF). CRF represents the cornea's ability to resist deformation in the presence of an external force.[49]

Goldmann applanation tonometry (Haag-Streit AG, Koeniz, Switzerland) is still recognized as the gold standard for measuring IOP in glaucoma patients and it was performed in all patients at all Visits. The ORA takes the CH and CRF

measurements into account to generate the corneal-compensated intraocular pressure (IOPcc) value, which is meant to be less affected by corneal biomechanical properties than the Goldmann-IOP.[29,49] The IOP bias is estimated as the difference between IOPcc and Goldmann-IOP (IOPcc – Goldmann IOP). A positive bias value indicates that the IOPcc is larger than the Goldmann-IOP, meaning that Goldman tonometry underestimated IOP in comparison to the IOPcc.

Four ORA measurements were taken per eye and the mean value was recorded for each parameter (CH, CRF and IOPcc). Central corneal thickness (CCT) was measured by ultrasound pachymetry (DGH Technology, INC, Exton, PA) and the average of three measurements was recorded. All study measurements were performed by the same trained observer (RM), with the same equipment and at the same time of day. All of the equipment needed for this research was calibrated prior to each use.

**Statistical Analysis**

The hypothesis of no effect regarding the discontinuation of PGA on the biomechanical properties was examined by a linear mixed-effect model using the nlme package in R. Random-effects and was defined on two levels: the patient (level-1) and the eye within each patient (level-2). Those random-effects were added to the model to account for the intra-individual variance due to the repeated-measure design. Age was also included in the model as a covariate. Contrasts between the eyes and times were estimated using adjusted p-values to control for familywise error rate using multcomp package in R.[24]

## 3.6 Results

Seventy eyes of 35 patients (16 males and 19 females) with open angle glaucoma were recruited. The mean age (± Standard Deviation) was 69.0.5 ± 9.3 years (range 50 to 85 years). The mean spherical equivalent was +0.25 ± 3.75 D (range -6.00 to +4.25 D). At the time of enrollment, patients were on PGA for 4 ± 2 years (range 1 to 7 years). The topical PGA used included: bimatoprost (Lumigan RC, Allergan Inc, Markham, ON, Canada) (8 patients); latanoprost (Xalatan, Pfizer Canada Inc, Kirkland, QC, Canada) (15 patients) and (APO Latanoprost, Apotex Pty Ltd, Macquarie Park, NSW) (1 patient); and travoprost (Travatan Z, Alcon Canada Inc, Mississauga, ON, Canada) (11 patients).

**Corneal Hysteresis**

A statistically significant increase in CH was found between Visit 1 (on PGA) and Visit 2 (no PGA) in the study eyes (Figure 11A), with mean (±Standard Error) values of 8.98 ± 0.29 mmHg and 10.35 ± 0.29 mmHg, respectively, corresponding to a mean increase of 1.37 ± 0.18 mmHg ($p < 0.001$). A significant reduction of 1.25 ± 0.18 mmHg ($p < 0.001$) was also observed between Visits 2 and 3, with a final mean CH value of 9.09 ± 0.29 mmHg. In addition, a statistically significant difference between the study and control eyes was only observed at Visit 2 (1.01 ± 0.23 mmHg; $p < 0.001$) and not at Visits 1 and 3.

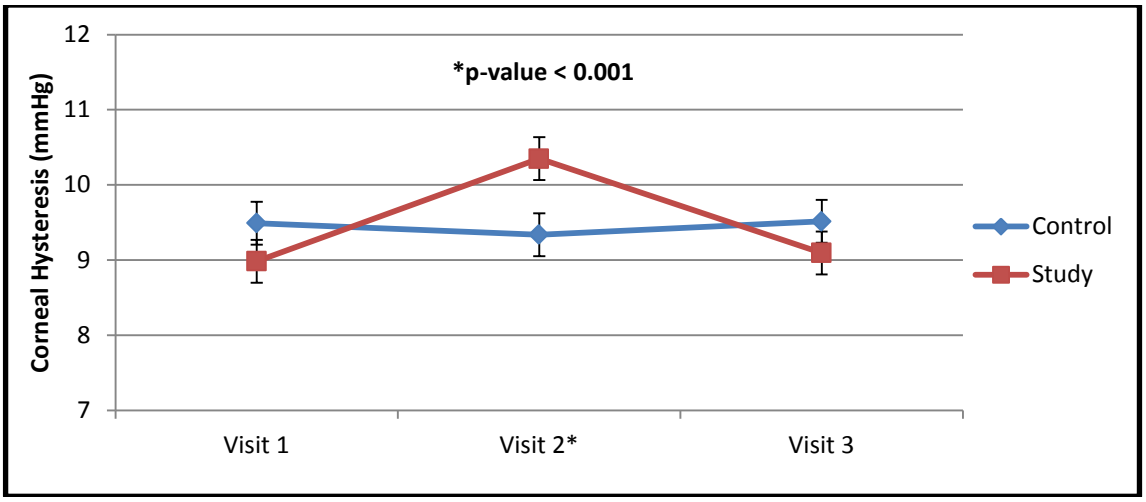


**Figure 11A: CH Expected Mean in mmHg (± s.e.) across time for each eye**

**Corneal Resistance Factor**

A statistically significant increase in CRF was found between Visits 1 and 2 in the study eyes (Figure 11B), with mean values of 10.23 ± 0.34 mmHg and 11.71 ± 0.34 mmHg, respectively. CRF was then reduced by 1.90 ± 0.21 mmHg ($p < 0.001$) between Visits 2 and 3, with a final mean CRF value of 9.81 ± 0.34 mmHg. A statistically significant difference between the study and control eyes was only observed at Visit 2 (1.46 ± 0.23 mmHg; $p < 0.001$).

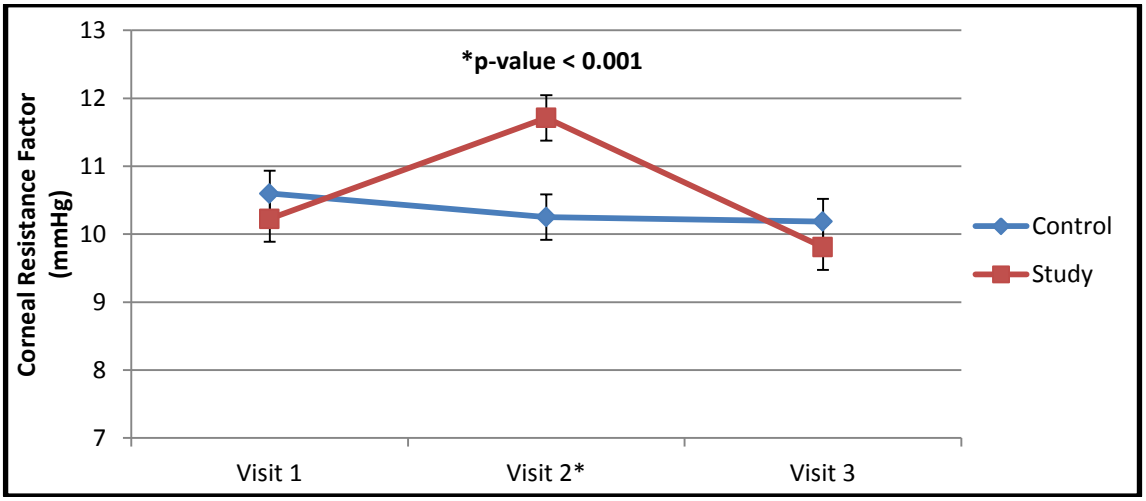


**Figure 11B: CRF Expected Mean in mmHg (± s.e.) across time for each eye**

**Central Corneal Thickness**

A statistically significant increase in CCT was found between Visits 1 and 2 in the study eyes (Figure 11C), with mean values of 541.83 ± 7.27 µm and 551.91 ± 7.27 µm respectively, corresponding to a mean increase of 10.09 ± 0.94 µm ($p < 0.001$). CCT then decreased by 9.40 ± 0.94 µm ($p < 0.001$) between Visits 2 and 3, with a final mean value of 542.51 ± 7.27 µm. A difference between the study and control eyes was only recorded at Visit 2 (11.26 ± 1.79 µm; $p < 0.001$).

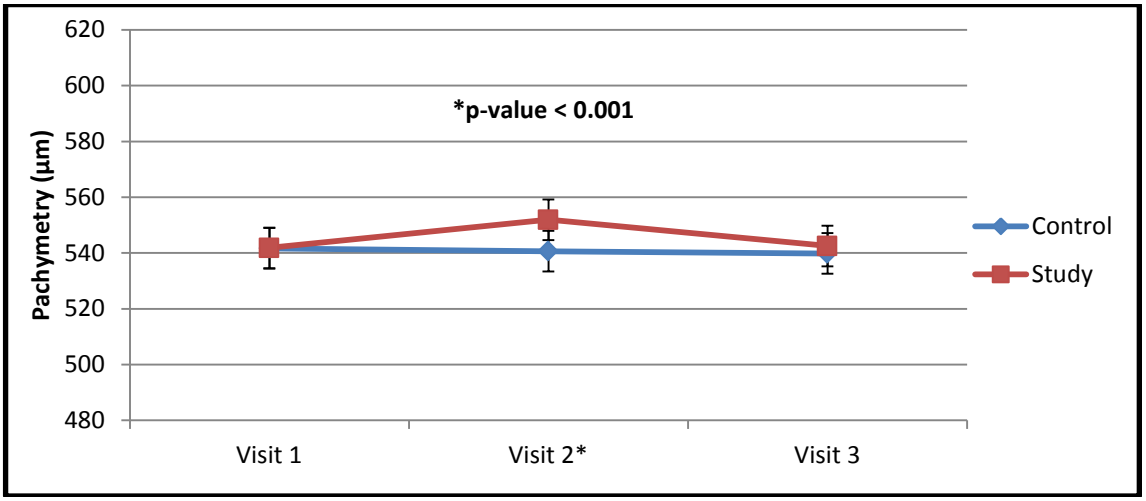


**Figure 11C: CCT Expected Mean in µm (± s.e.) across time for each eye**

**Goldmann Intraocular Pressure**

Similarly, a significant increase in IOP was observed between Visits 1 and 2 in the study eyes (Figure 11D), with mean values of 15.37 ± 0.54 mmHg and 18.37 ± 0.54 mmHg respectively, corresponding to a mean increase of 3.0 ± 0.49 mmHg ($p < 0.001$). A significant reduction of 2.83 ± 0.49 mmHg ($p < 0.001$) was observed between Visits 2 and 3, with a final mean IOP value of 15.54 ± 0.54 mmHg. The control and study eyes only differed at Visit 2 (1.91 ± 0.49 mmHg; $p < 0.001$), confirming the effectiveness of PGA treatment.

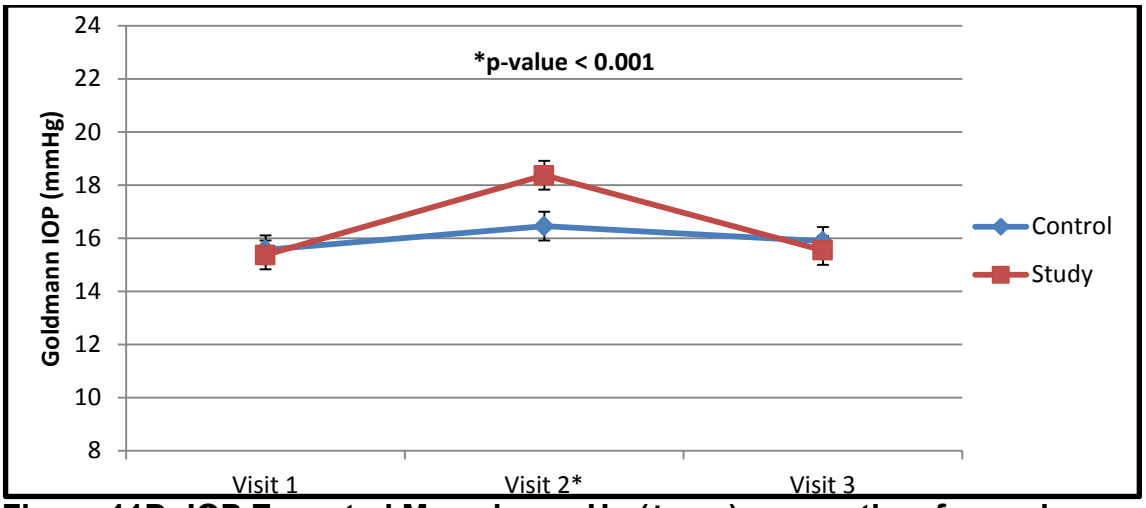


**Figure 11D: IOP Expected Mean in mmHg (± s.e.) across time for each eye**

**IOP Bias**

At Visit 1, the IOP bias (IOPcc – Goldmann IOP) was similar in both groups, all eyes at that time being on long term PGA medication, with mean values of 4.1 ± 0.54 mmHg in the control eyes and 4.8 ± 0.54 mmHg in the study eyes. At Visit 2, after a 6 week washout of PGA, the IOP bias in the tested eye was reduced to 1.6 ± 0.54 mmHg ($p<0.001$), meaning that underestimation of IOP by Goldmann tonometry was significantly less than at Visit 1 (Figure 11E). The difference in mean IOP bias between the study and control eyes at Visit 2, however, did not reach statistical significance ($p= 0.124$). A marginally significant increase of 1.53 ± 0.60 mmHg ($p = 0.055$) was observed between Visits 2 and 3 in the study eyes, with a final mean IOP bias value of 3.10 ± 0.54 mmHg in the study eyes and 2.8 ± 0.54 mmHg in the control eyes. The IOP bias in control eye was changed as well during the visits. The IOP bias is estimated as the difference between IOPcc – Goldmann IOP. The mean IOP and IOPcc in control eyes at the Visit 1 was 15.571 mmHg and 19.714 mmHg respectively with IOP bias 4.143 mmHg. As shown at Figure 11D the Goldmann IOP increased at Visit 2 up to 16.457 mmHg

that caused decreasing in IOP bias up to 2.792 mmHg. The Goldmann IOP at Visit 3 decreased up to 15.886 mmHg but because of decreasing in IOPcc at Visit 3 the IOP bias remained unchanged. The curve of the control eye during the visits at Figure 11E deviated depend on these changes in GAT IOP and IOPcc. The changes in control eye during visits confirmed that these processes not understood well and further researches should be conducted with more precise IOP control. It can be achieved by using systemic or topical IOP lowering medications that control IOP and do not influence biomechanical properties of the cornea.

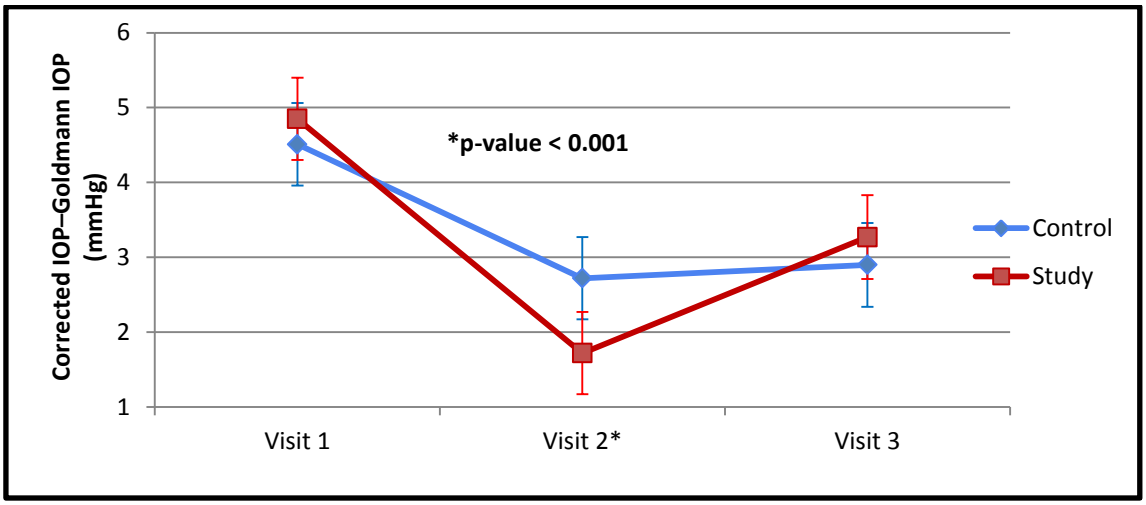


**Figure 11E: IOP Bias (Corrected IOP – Goldmann IOP) Expected Mean in mmHg (± s.e.) across time for each eye**

### Visual Field Mean Defect and CH

We then tried to determine if a low CH was associated with signs of more severe glaucoma damages among our open-angle glaucoma patients treated with PGA. When considering all eyes on PGA at the time of the first visit, no association was found between VF damage and CH. However, when considering only eyes with more advanced glaucoma (defined by a VF MD < -6.0 db and represented in

red Figure 12), a significant positive correlation was observed between VF MD and CH (B = 0.65; p = 0.003). A lower CH was associated with a more negative visual field MD value.

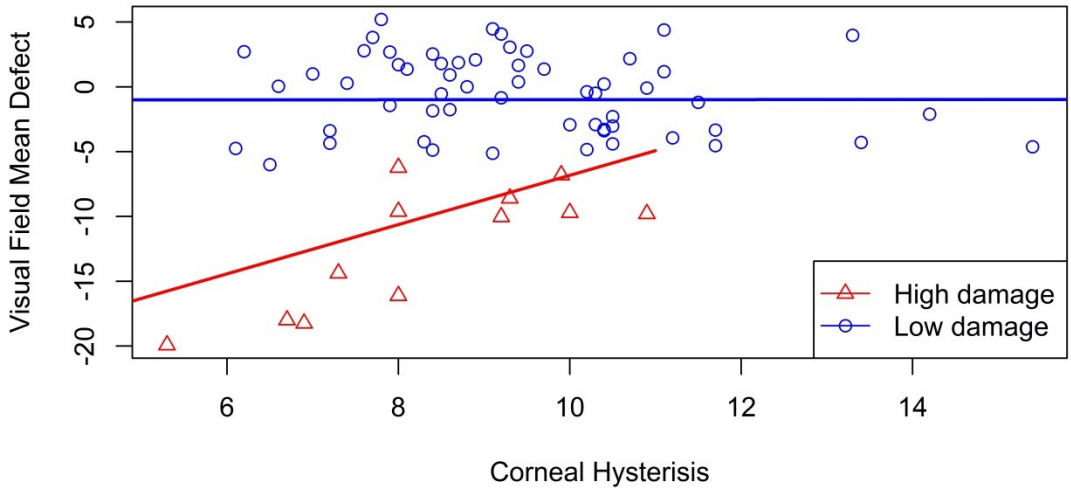


**Figure 12: Visual Field Mean Defect on CH by glaucomatous damage**

## 3.7 Discussion

This study demonstrated the influence of PGA on the biomechanical properties of the cornea and IOP in glaucomatous eyes. It was shown that chronic uses of PGA not only reduce IOP but also lowered CH, CRF and CCT. To our knowledge, this is the first study that supports the hypothesis that PGA reduces CH in glaucomatous eyes. This observation is clinically relevant as a low CH is known to be associated with underestimation of IOP[5, 51, 52] and with a greater susceptibility to glaucoma-related damage. A low CH is recognized as a predictive factor for visual field progression.[53,54] In the context of our study, this

reduction in biomechanical properties of the cornea induced by the administration of topical PGA was reversible as a 6-week period of abstinence of PGA use resulted in a statistically significant increase in CH, CRF and CCT.

One of the strengths of our study was the use of a repeated-measures design. This design is much more powerful in detecting significant differences across the pair of eyes and across times because each subject is his own control. The strength of this study also was that no dropouts were recorded, resulting in a well balanced design.

***PGAs & CH***

Studies regarding the effects of PGA on CH have already been conducted; however, these studies compare the effects of PGA on CH to untreated baselines. Two recent studies by Agarwal et al. and by Tsikripis et al. have found a sustained increase in CH following the administration of PGA medication. Both studies conducted their experiments on open-angle glaucoma patients selected using an inclusion criteria similar to those used in our study and with a similar age range.[55, 56]

One of the main methodological differences involved the duration of PGA therapy at the time of the study involvement. While the two studies began from untreated baseline[55, 56], our participants were previously undergoing daily PGA treatment for 4 ± 2 years prior to the start of the study.

Upon further analysis of the results collected from Tsikripis et al., we discovered that the magnitude of increase in CH is not significant if the mean CH differences are measured with respect to the CH value obtained after 1 year of treatment.[56]

*CH and CRF are related, but are different measurements*

In a study conducted by Shah et al., it was found that CH and CRF are significantly correlated and have a correlation coefficient of 0.8. Despite the relationship, the study also illustrated that CH and CRF do not reflect the same parameter.[57] This finding justified our examination of the effects of PGA on CRF. Previous studies have examined the effects of PGA on CRF; however, the literature contains mixed conclusions. A study conducted by Detry-Morel et al., in which 75 of 108 glaucomatous eyes and 9 of 22 ocular hypertensive eyes were treated with PGA prior to the ORA measurements, reported a decrease in CRF following PGA use.[58] This is in agreement with the findings of our study. However, the previously mentioned study conducted by Tsikripis et al. reported no change in CRF following PGA treatment.[56] The results may be explained by the timing of data collection during the period of PGA treatment. In addition, based on the correlation between CH and CRF, the manipulations affecting one parameter may stimulate a similar response in the other. Figure 13 shows the comparative characterization of the researches related to impact of topical PGA on the biomechanical properties of the cornea.

***Visual Field Mean Defect and CH***

As previously mentioned, the biomechanical properties of the cornea play crucial roles in glaucoma-related damages and progression. While CCT only represents one parameter of the corneal properties, the CH is a direct measurement of the biomechanics of the whole eye.[59] Previous studies found that CCT is associated with visual field loss and progression of OHT to glaucoma.[54,60] Moreover,

Congdon NG et al. showed that glaucomatous visual field loss is associated with low CH and is independent of CCT.[54]

| Author | Aim of Study | # of eyes /patients | Follow-up | Pts. ages groups | Refraction | Rx general | Diagnosis | Rx oc. | Investigation | Analysis | Conclusion(s) of the study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Kim 2011** | Retrospective PGA vs CCT | 166 eyes | 24 months | 43-70 | No | No | First diagnosis NT OAG | Latanopr. | U/S, GAT | t-test | Long-term use of latanoprost may decrease the CCT in patients with NTG. |
| **Sen 2008** | Prospective PGA vs CCT | 188 eyes | 6-12-24 months | 45-68 | No | Exclude steroid | First diagnosis POAG, NTG, OHT | Latanopr., Bimatopr. | U/S, GAT | t-test, Wilcoxon | A significant reduction in CCT was observed at the 6, 12 and 24 months with latanoprost and bimatoprost. |
| **Arcieri 2008** | Prospective PGA vs CCT | 68 eyes | 4 weeks | 39-76 | No | No | First diagnosis POAG, NTG, OHT | Latan. Bimatop. Travopr. | U/S, GAT | t-test | Latanoprost, travoprost and bimatoprost had no statistically significant effect on the blood-aqueous barrier of phakic patients with POAG or OHT. Bimatoprost may be associated with a clinically irrelevant reduction in mean CCT. |
| **Zhong 2012** | Prospective PGA vs CCT | 69 eyes | 6 and + months | 37-65 | No | No | First diagnosis POAG, NTG, OHT | Latan. Bimatop. Travopr. | U/S, GAT | t-test | Topical therapy with prostaglandin analogues is associated with CCT reduction. Latanoprost, travoprost, and bimatoprost have a similar effect on CCT. |
| **Agarwal 2012** | Retrospective CH vs IOP and CCT vs IOP | 109 eyes | 24 months | 40-70 | No | No | POAG | PGA, no specify | ORA, U/S, GAT | t-test, ANOVA | Although CH is influenced by IOP, baseline CH is independently associated with the magnitude of IOP reduction with PGA therapy.<br><br>PGA:<br>**> CH increased**<br>**> predictive value: lower baseline CH yield greater lowering IOP** |
| **Detry-Morel 2011** Small attempt of CH/CRF vs PGA | Prospective CH/CRF, ORA and GAT IOP, CCT characteristic in different groups of Pts | 154 eyes | 9 months | 34-86 | +3.00D to -3.00D | No | First diagnosis POAG, OHT, NTG, NP | Different groups of Pts + PGA | ORA, GAT, U/S | Wilcoxon, Chi-square test, rank-sum test, Kruskal-Wallis analysis, SPSS software | **Glaucoma patients** have very probably **distinctive biomechanical** properties of the cornea compared to OHT and controls. Both parameters may be influenced by many parameters and can otherwise behave independently but they do not replace corneal pachymetry assessment in the current state of knowledge. Our data supports the hypothesis that a low corneal hysteresis could be considered as a risk factor for underestimation of IOP which may have potential implications in the management of glaucoma patients and glaucoma suspects. Simultaneously, we could confirm that the ORA provide an IOP measurement that is independent from central corneal thickness. Although CRF appeared only to be influenced by topical medications, further stratified multivariate prospective studies are needed to confirm these data, and especially to precise the exact influence of antiglaucoma medications on the biomechanical properties of the cornea.<br>PGA:**CH unaffected**<br>**CRF decreased** (like with other topical Rx) |
| **Bafa 2011** | Prospective PGA vs CCT | 129 eyes | 0-3-6-9-12-18-24 months | 60-80 | No | No | First diagnosis POAG | Latan. Bimatop. Travopr. B-blockr | U/S, GAT | t-test, Wilcoxon, STATA | A slight but significant increase in CCT was recorded in the prostaglandin group. This was not the case in B-blockers group.<br>[[so still a controversy?]] |
| **Birt 2012** | Prospective PGA vs CCT | 75 eyes | 2-6-12-24 weeks | 52-72 | No | No | First diagnosis POAG, OHT | Latan. Bimatop. Travopr. | U/S ?IOP | ANCOVA | They found a statistically significantly association between a lower mean IOP and a thinner cornea when baseline IOP is controlled for. The magnitude of the relationship is small but may be clinically significant in patients with either very thin or very thick corneas. |
| **Bolivar 2011** | Prospective PGA vs CCT Rabbit | 12 eyes | 1 month | n/a | n/a | n/a | Rabbit | Travopr. | U/S ?IOP | Wilcoxon test | Rabbit corneas treated with topical travoprost show a different strain response to acute increases in IOP than control eyes. |
| **Hatanaka 2009** | Prospective PGA vs CCT | 146 eyes | 8 weeks | 47-85 | No | No | First diagnosis POAG | Latan. Bimatop. Travopr. | U/S, GAT | t-test, ANCOVA | Topical therapy with prostaglandin analogs and bimatoprost is associated with CCT reduction over a period of at least 8 weeks. |
| **Harasymowycz 2007** | Prospective PGA vs CCT | 379 pts | 6 weeks | 18-90 | No | No | First diagnosis POAG, OHT | travoprost | U/S,GAT | S-PLUS software, t-test | Treatment with travoprost decreased IOP significantly and was associated with CCT thinning, which had little or no effect on actual IOP decrease. |
| **Brandt 2004** | Prospective PGA vs CCT, B-blocker vs CCT | 817 eyes | 12-60 months | 47-70 | No | Yes | First diagnosis POAG, OHT | PGA and β-blocker | U/S, GAT | X2-test, t-test | Impact of CCT on IOP response to ttx:<br>Individuals with thicker corneas had smaller measured IOP responses to ocular hypotensive medication than those with normal or thin corneas. |
| **Sawada 2012** | Prospective PGA vs CCT | 42 pts | 1-3-4-6 months | 26-74 | No | No | First diagnosis POAG, NTG | Latan. Travopr | U/S, GAT | ANOVA, Wilcoxon, U test, Stata software | Travoprost has similar effect as latanoprost in reducing the IOP in glaucoma patients with relatively low IOPs. The use of prostaglandin analogs can decrease the CCT, and this change should be considered when the IOPs obtained by GAT are analyzed. |
| **Schlote 2009** | Prospective PGA vs CCT | 136 eyes | 6-12 months | 34-88 | No | No | First diagnosis POAG, NTG, OHT | travoprost | *OLCR, GAT | MEDCALC 9.3.6.0 software | Topical therapy with the prostaglandin derivate travoprost is accompanied by a significant reduction of CCT within one year (6 months) of treatment.<br><br>***Non-contact** Ocular Low Coherence Reflectometry, Haag-Streit, Switzerland |
| **Tsikripis 2013** | Prospective PGA vs CH, CRF, CCT | 108 eyes | 0-6-12-18-24-30-36 months | 45-80 | No | No | POAG | 66 – latanoprost 42 – latan+timolol | U/S, GAT, ORA | Kolmogorov–Smirnov and Shapiro–Wilk statistics | It appears that under local PGA treatment, IOP values decreased and CH and CCT significantly increased, whereas CRF did not. |

**Figure 13: The comparative characterization of the researches related to topical PGA.**

The association between CH and optic disc surface compliance described by Wells A.P. et al. suggests that a floppy cornea is associated with floppy lamina

cribrosa and peripapillary connective tissue structures.

Hysteresis refers to the ability of the ocular connective tissues to dampen pressure changes rather than having characteristics of floppiness or rigidity.[59] The data collected from our study presented an association between CH and visual field damages with a positive correlation between low CH and more negative MD. Thus, the concept that CH not only represents corneal biomechanical properties but also the biomechanical properties of the entire eye was confirmed in this study.

***Mechanism of Action of PGAs***

The mechanism by which PGA affect CH is unclear; however, it is has been suggested that PGA enhances collagen and extracellular matrix degradation by disturbing the normal levels of matrix metalloproteinases (MMPs) and tissue inhibitor metalloproteinases (TIMPs).[61]

In a study where participants were on PGA medication for a minimum of 1 year prior to the start of the study, the chronic use of PGA resulted in significant increases in the immunoreactivity of matrix metalloproteinase-1 (MMP-1) and matrix metalloproteinase-9 (MMP-9) in the epithelium and corneal stroma. MMP-1 is classified as a collagenase. It functions to degrade collagen types I, II and III as well as other molecules both belonging and not to the ECM. MMP-9 is classified as a gelatinase and can degrade collagens and gelatins.[62] In addition, a significant reduction in the immunoreactivity of the tissue inhibitor metalloproteinase-1 (TIMP-1) was observed in the same corneal compartments. MMPs degrade the collagen and the extra-cellular matrix while TIMPs protect

from excessive degradation by inhibiting the MMPs.[8, 42, 61] The overexpression of MMPs and underexpression of TIMPs caused by the prolonged use of PGAs results in an enhanced degradation of the corneal collagen and extracellular matrix.[61] Corneal hysteresis is dependent on the collagen and extracellular matrix composition of the cornea.[50] Thus, it is possible that the loss of collagen and extracellular matrix results in a reduction in CH.

A possible alternative can be explained by analyzing (Figure 10). Due to the discovery of the same PGA receptors on the cornea as on the trabecular meshwork, it is possible that PGA decrease CH and CCT by a similar mechanism of action as IOP.[41, 42] These processes lead to molecular changes in the cornea and can be divided into 2 categories based on a time-action parameter: **(i)** Short time-action processes including compression and degradation of the extracellular matrix, increased lysis of extracellular connection between collagen molecules or fibrils and increased spacing between the collagen fibrils and muscle bundles. Also part of this category is the reorganization of the cytoskeleton by activation of a molecular transduction cascade (increase in transcription of matrix metalloproteinases),[7, 8, 63] corneal stromal receptors such as P2X7[9, 43-46, 64-66] and a platelet-activating factor receptor.[41, 42, 47, 48, 67] Processes of this category are easily reversible. **(ii)** Long time-action processes including increased apoptosis of stromal cells, decreased mRNA expression in the major components of the corneal stroma and decreased reproduction of collagen types I and V in the cornea by activation of corneal stromal receptors such as P2X7[9, 43-46, 64-66] and a platelet-activating factor receptor[41, 42, 47, 48, 67] that are possibly not reversible.

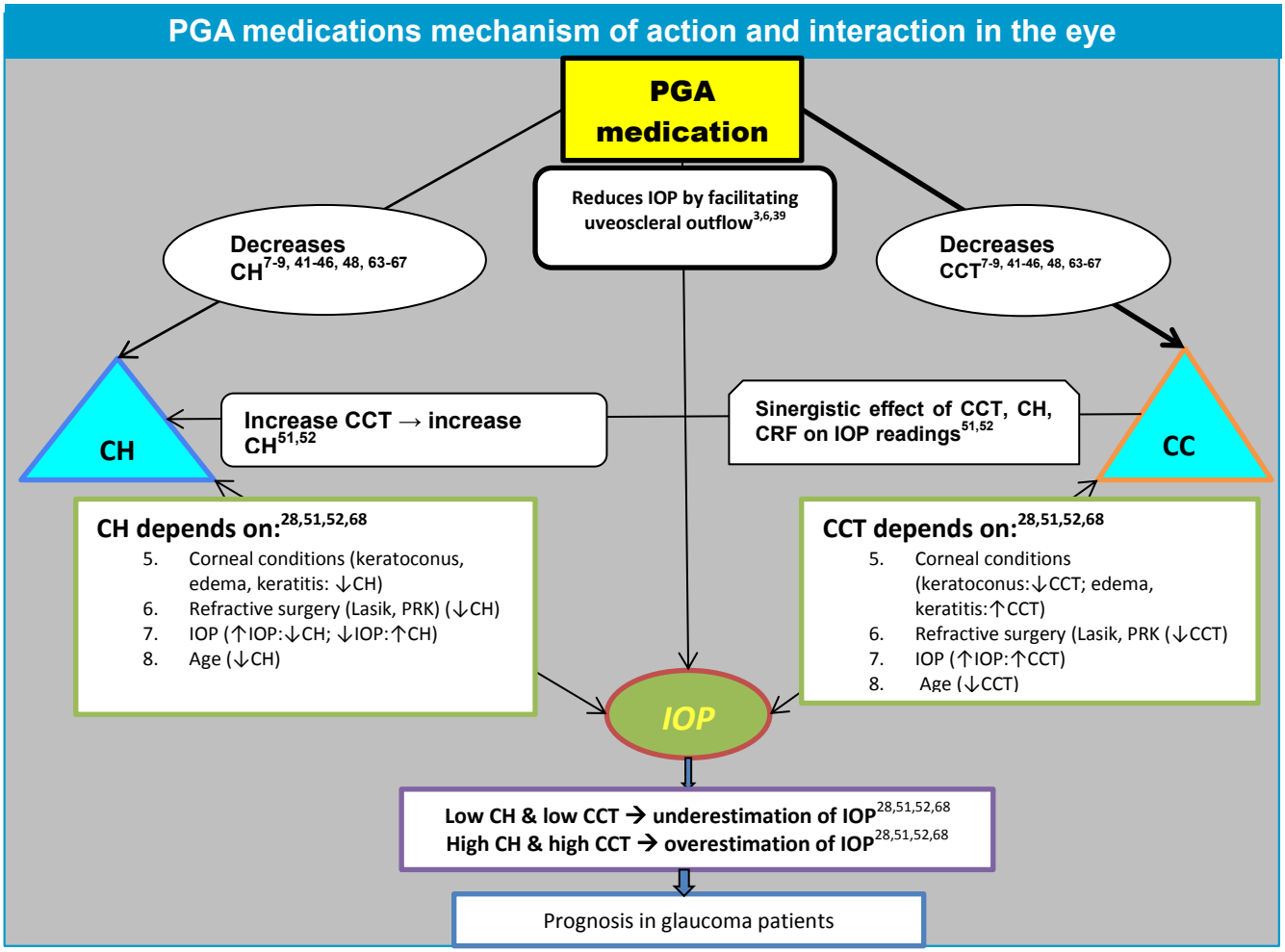


**Figure 10: The action and interaction of PGA medication in the eye**

### *Clinical Value of our Results*

The results collected in this study have clinical value as a low CH was shown to be associated with more glaucoma damages on visual field. It was also associated with a greater susceptibility to glaucoma damage.[53, 54] Furthermore, low CH in combination with low CCT is known to result in underestimation of IOP.[5, 51, 52] A reduction in CH renders the cornea less resistant to the applied force of the Goldmann applanation tonometer, resulting in the observed inaccuracies.[68] The reading of an underestimated IOP value may lead to the

implementation of insufficient treatment which may worsen the prognosis of glaucoma.

***The Importance of our Study***

This study provides a new understanding of glaucoma management and highlights the importance of corneal hysteresis in the therapy and prognosis of glaucoma. This study also suggests using the Goldmann applanation tonometer in combination with a device that considers the corneal biomechanical properties, such as the ORA, for the measurement of IOP.

## 3.8 Conclusions and Perspectives

Topical prostaglandin analogs reduce corneal hysteresis, central corneal thickness and intraocular pressure measurements in glaucoma patients. These changes in corneal hysteresis and central corneal thickness affect IOP readings and should be taken into account when evaluating IOP lowering response to PGA medications. To better understand the action of topical prostaglandins on the biomechanical properties of cornea, further research should be conducted. To discern an interaction between IOP and corneal hysteresis, further research should be conducted with intraocular pressure control. It can be achieved by using systemic medications that decrease IOP and do not influence biomechanical properties. It can also be achieved by using Pascal dynamic contour tonometry or a similar tonometer that does not depend on the biomechanical properties of the cornea.

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