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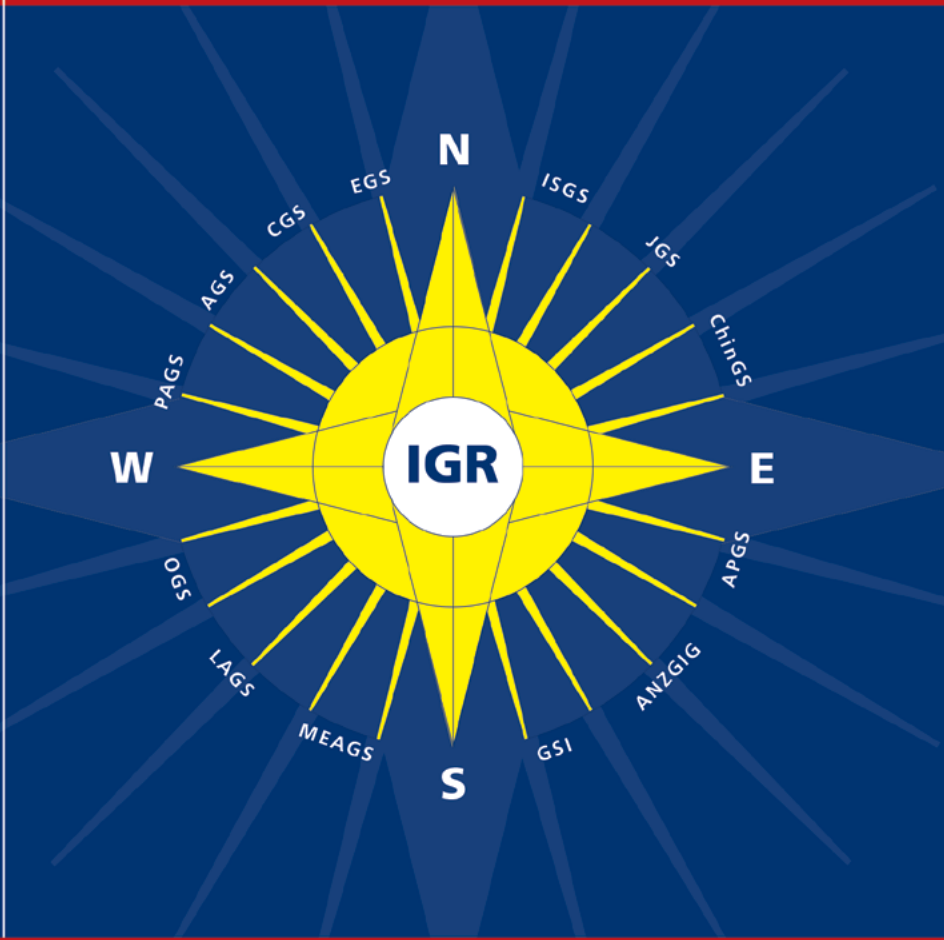

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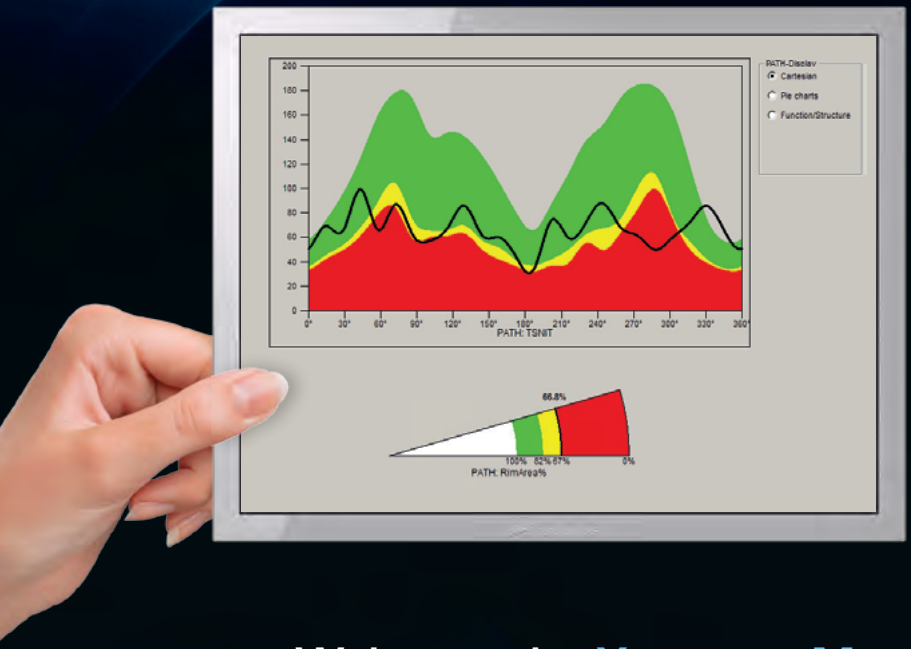

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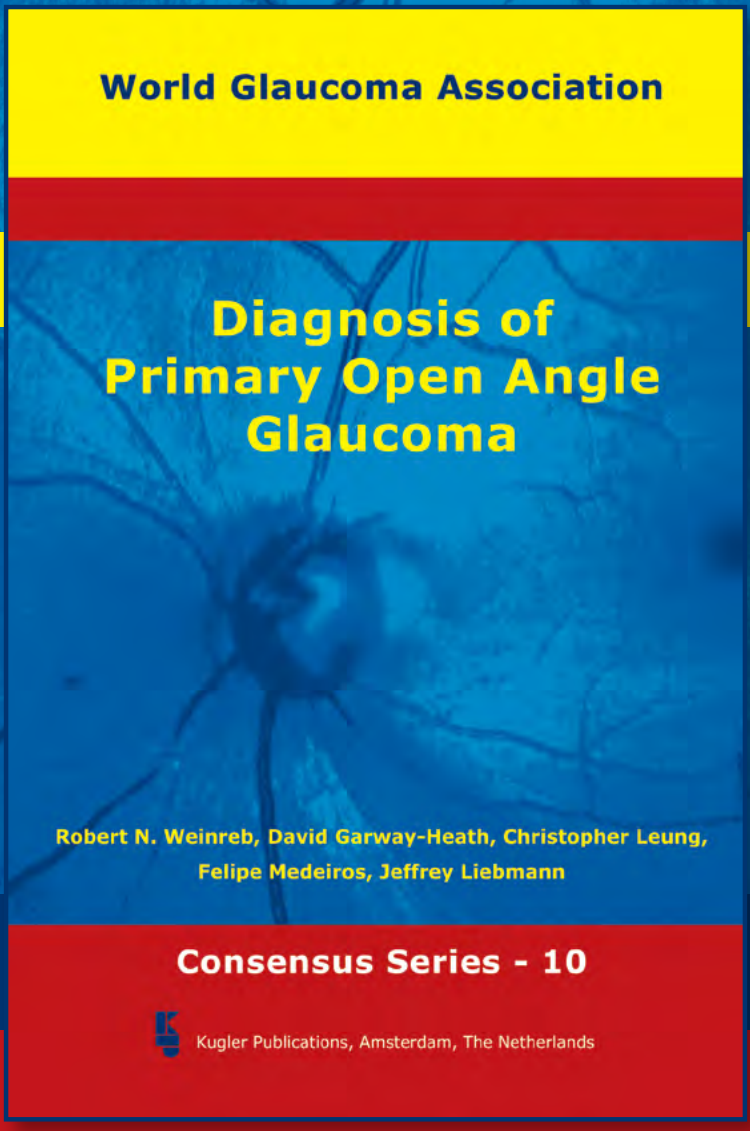

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Robert N. Weinreb, Chief Editor

71509 Vitamin B3 modulates mitochondrial vulnerability and prevents glaucoma in aged mice; Williams PA, Harder JM, Foxworth NE, Cochran KE, Philip VM, Porciatti V, Smithies O, John SW; Science 2017; 355: 756-760

Comments

Comment by Keith Martin, Cambridge, UK

Many patients with glaucoma continue to experience progressive visual loss despite treatment to lower their eye pressure and thus the development of new therapeutic strategies to protect the vision of such patients remains an important goal. The paper by Williams *et al.* describes the novel finding that a reduced level of nicotinamide adenine dinucleotide (NAD) in aged mice is an important risk factor for retinal ganglion cell loss. The researchers studied DBA/2J mice, a strain which develops an eye condition with similarities to pigmentary glaucoma. They found that dietary supplementation with nicotinamide, a precursor of NAD, or gene therapy to drive the expression of a NAD-generating enzyme [nicotinamide nucleotide adenylyltransferase 1 (Nmnat1)] could both effectively halt glaucomatous disease progression in these animals. **The strength of the neuroprotective effect obtained by manipulation of a single molecule was truly remarkable,** and the

additional observation that the higher dose of dietary nicotinamide also decreased eye pressure in the DBA/2J mice was also interesting, hinting at a possible dual mechanism of action.

Another interesting question that arises from this work whether the site of protective action by NAM and NMNAT1 is in the cell body, the axon or both. A nuclear site of action might be suggested by previous observations that DNA damage by PARP1 depletes nuclear NAD and NMNAT1, and is a nuclear enzyme that fails to protect injured axons in transgenic mice unless it is excluded from nuclei. However an axonal action would also appear conceivable and it would be interesting to explore this issue in future studies.

As for many studies in animal models of glaucoma, a key consideration is how generalizable the results are to human glaucoma

As for many studies in animal models of glaucoma, a key consideration is how generalizable the results are to human glaucoma. All models, including DBA/2J, have limitations and it is good that the authors also tested their therapeutic approach in a tissue culture model of axotomy and in a model involving intravitreal injection of TNF alpha. Replication of the results in other models would give increased confidence that these results could be relevant to humans, but the real answer will come from human clinical trials which would appear to be feasible and realistic. As a prelude to human studies, it will be important to determine if the very doses used in this proof-of-concept study are required to achieve a therapeutic effect.

Comment by Louis R. Pasquale, Boston, MA, USA

New approaches to protect the optic nerve from glaucomatous degeneration are always welcome. Williams and coworkers perform a rigorous set of experiments in a mouse model of glaucoma (the DBA/2J mouse) characterized by variable-onset but age-related iris depigmentation, immune deviation, elevated intraocular pressure (IOP) and optic nerve degeneration. The RNA sequence expression in RGCs of DBA/2J mice at nine months, a time when there is elevated IOP but no optic nerve damage, demonstrates an increase in mitochondrial gene expression suggestive of a cellular stress response. In DBA/2J mice, but not in controls, these biochemical changes were accompanied by structural reductions in mitochondrial cristae surface area. Cristae are folds in the inner mitochondrial membranes that allow electron transport and ATP formation to occur. The conversion of NADH to NAD+ is a key upstream step in the generation of a hydrogen ion gradient that leads to ATP formation. The authors found that an early reduction of NAHD and NAD+ in RGCs represents

an important biochemical signature of aging that was present in both DBA/2J mice and controls. The authors therefore hypothesized that reduction of cellular respiration served as a sensitizer to make RGCs vulnerable to glaucomatous degeneration. Mice were treated with 0.55g/kg/day nicotinamide (NAM), a precursor of NAD, in the drinking water at either six months or nine months of age and followed until one year of age. Approximately 50% of control eyes exhibited > 50% axon loss at 12 months, while ~22% of treated eyes exhibited such loss when NAM was started at nine months of age. When the NAM dose was increased to 2g/kg/day the optic nerve protection was even more pronounced. Furthermore. NAM restored the normal ultra-structural features of mitochondrial cristae. These neuro-protective effects were achieved without lowering IOP. Similar neuro-protective effects were seen when RGCs from DBA/2J mice over-expressed nicotinic acid mononucleotide adenyltransferase, an enzyme that generates NAD.

These experiments are certainly elegant, but there are a few issues to consider. The animals were sex-matched but the percentage of males and females was not provided. While this may seem like a minor point, the subsequent performance of RCTs requires such preclinical knowledge. Second, it would be interesting to carry out comparable experiments in the micro-bead glaucoma model. **While there is probably immune involvement in human open angle glaucoma, the immune alterations in the DBA/2J model are likely more pronounced.**

Their results support the therapeutic use of NAM in glaucoma; yet, they used NAM doses that are probably not feasible in humans

Finally the authors make a strong statement that their results support the therapeutic use of NAM in glaucoma; yet, they used NAM doses that are probably not feasible in humans. NAM, also known as niacin or vitamin B3, can produce cystoid macula edema at doses of three to six g/day, but this side-effect has been reported with lower doses.[1,2] Systemically niacin commonly produced facial cutaneous flushing, a side effect that could curtail its use. The lowest NAM dose that was associated with a statistically significant neuro-protective effect was not reported. Overall structural and functional tests suggest that these doses enhance retinal integrity but there is no mention of whether idiopathic retinal toxicity was noted in any of the mice that received NAM.

Overall, we hope that the authors find ways to translate their findings to glaucoma patients. It should be noted that while the favorable effect of NAM on serum lipid profile is unquestioned, it failed to have definitive effects on cardiovascular endpoints or all-cause mortality in a recent meta-analysis of 13 clinical studies.[3] Perhaps, investigators can leverage pre-existing clinical and epidemiological data to test whether NAM intake favorably alters the course of glaucoma.

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Comment by Harry Quigley, Baltimore, MD, USA

This report is deals with an important and timely subject. Readers should assess several important aspects of its methods and conclusions. First, NAM treatment may behave differently in DBA/2J mice and it should be tested in other glaucoma models for three reasons.

First, NAM treatment may behave differently in DBA/2J mice and it should be tested in other glaucoma models

1. **While the authors claim to study "how increasing age and high IOP interact to drive neurodegeneration", the study does not test age as a variable**. DBA/2J mice that develop abnormal IOP lose retinal ganglion cells (RGC) around 6-12 months of age. No data are presented here comparing younger to older animals with similar IOP exposure – that requires comparing effects of IOP elevation and RGC protection in young compared to old mice. We, and others, have studied young versus older mouse RGC susceptibility to experimental IOP elevation, showing different effects of age in different strains of mice.

2. DBA/2J mice have dramatic degeneration of anterior ocular structures and the consequent inflammation is integral to this model, but not shared by human glaucoma or other rodent models that elevate IOP. The fact **that higher doses of NAM prevented IOP elevation suggests that the inflammatory/degenerative features of DBA/2J may lead to different outcomes from other glaucoma models if the treatment blocks its unique features, but not general RGC protection pathways.**

3. DBA/2J mice have a highly variable IOP profile and variable time to onset of RGC damage. Thus, it is difficult to study the time course of neurodegeneration, which is better modeled in other high IOP models (*e.g.*, laser treatment, bead injection or hypertonic saline exposure), where the injury initiation is clearly defined in time. If NAM treatment is confirmed as

useful in additional models, it will be possible to dissect its true pathway of protection. We cannot yet know from the present work whether the localization of the beneficial effect, if any, is at the cell body, the axon, both or neither.

There are other **methodological issues**.

1. Mouse RNA data were divided into four groups with the claim that "As disease progressed, there was an increase in transcript abundance that was most pronounced for mitochondrial reads." **No evidence was provided that the transcript groups had different amounts of RGC loss or were matched to some level of progression**, only that they could be segregated into groups.

2. Axon loss was assessed only qualitatively and the methods were insufficiently described in order to allow determination if there was any real effect. Paraphenylenediamine (PPD) was described as a "sensitive stain for damaged axons", while in fact it non-specifically labels many aspects of nerve tissue and is not specific for injury, which was judged subjectively. If axons are dead and leave no clear debris, which is often the case in mice, the method mistakes the nerve as normal. More accurate methods to assess axon loss are demonstrated in many papers, involving sensitive counts of the actual axon density in randomized images multiplied by nerve area. Quantitative methods show that the variability among mice in axon loss often requires 30 mice or more in a group to show effects at the level seen here, where there were only 8 mice in a group. In the critical Fig 2 C, one cannot tell which groups are being compared.

3. **The claim that there is a "loss of axonal transport" with NAM treatment is not documented by any quantitative data, nor a presentation of the method used.**

Prior to this paper, there were already many investigators publishing important links between mitochondrial function and glaucomatous damage. This paper will hopefully stimulate more interest in that field to corroborate or modify its findings.

Comment by Derek Welsbie, La Jolla, CA, USA

DBA/2J (D2) mice develop a form of chronic glaucoma with iris abnormalities, elevated intraocular pressure (IOP) and retinal ganglion cell (RGCs) loss.[1] In order to better understand the early molecular events responsible for IOP-dependent RGC loss, Williams *et al.* used fluorescence-activated cell sorting to isolate RGCs from D2 mice with or without elevated IOP.[2] The experimental group used nine-month-old D2 mice with elevated IOP, but before the onset of frank RGC loss. Controls animals included D2 mice at four months, before the onset of elevated IOP, and nine-month-old congenic D2 mice with a wildtype *Gpnmb* allele (D2-*Gpnmb*$^{+}$) that do not develop elevated IOP. They then used RNA sequencing to analyze and compare the transcriptomes of the various RGC populations. The results showed that genes associated with mitochondrial stress responses and oxidative phosphorylation

were differentially expressed, suggesting that mitochondrial dysfunction could be an early pathophysiologic event in response to elevated IOP. The authors went on to measure the level of the mitochondrial metabolite, nicotinamide adenine dinucleotide (NAD^+), in D2 and control D2-*Gpnmb*$^+$ mice and, in contrast to the above, found that whole-retinal NAD^+ levels declined with age, *independent* of the presence of elevated IOP.

In other tissues, NAD^+ levels are known to decrease with age and there is a rich body of literature on the role of NAD^+ (and a class of NAD^+-dependent enzymes known as sirtuins) in aging and neurodegeneration.[3] Moreover, it is known that NAD^+ depletion (and reduced sirtuin activity) can affect mitochondrial function and turnover and that genes (*e.g.*, TBK1 and optineurin) that affect mitochondrial turnover can cause rare familial forms of glaucoma. Finally, NAD^+ metabolites play a critical role in the SARM1-dependent genetic program responsible for axon degeneration after injury[4] and axon injury at the optic nerve head is a key causal event in D2 glaucoma.[5] Given this constellation of findings, the authors postulated that reduced NAD^+ levels in aged RGCs might be necessary for elevated IOP to cause mitochondrial dysfunction, leading to axon degeneration and cell death.

To test this hypothesis, the authors turned to a biosynthetic precursor of NAD^+ known as nicotinamide (NAM) or vitamin B_3, which when given exogenously, can raise the levels of NAD^+. Of note, vitamin B_3 can also refer to a structurally-related precursor called niacin (nicotinic acid). Starting at either six months (before the onset of increased IOP) or at 9 months (after the onset of increased IOP), 550 or 2000 mg/kg/day of NAM was orally administered to D2 mice and the expected increase in retinal NAD^+ concentrations was verified. They then examined eyes at 12 months, when the neurodegeneration is typically manifest, and showed a dose-dependent neuroprotection that was similar regardless of when the therapy was initiated. At the molecular and subcellular level, NAM supplementation reversed the gene expression changes normally seen with aging and glaucoma and improved mitochondrial morphology. At the cellular and tissue level, NAM led to preservation of RGC cell bodies, synaptic marker staining, electrophysiological activity and axons connected to the lateral geniculate nucleus/superior colliculus. The high (but not low) dose had an additional effect of blunting the IOP increase in D2 mice, confounding a direct effect of high dose NAM on RGCs. Finally, the authors were able to achieve *near-complete* protection by combining the low dose of NAM with overexpression of nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 (NMNAT1), an enzyme that converts NAM to NAD^+.

Although it can be difficult to compare mouse and human dosing, the equivalent NAM dose in humans would far exceed the lethal concentration

Although the authors tested other models of RGC injury, it will be **important to test this strategy in other animal models of glaucoma.** Indeed, Wallerian degeneration slow, WLD^S (a chimeric protein that includes NMNAT1) acts in a similar manner but was *unable* to provide sustained RGC protection in the rat laser model of glaucoma.[6] Moreover, it may be the case that the pathway is more complicated as NMNAT1 can inhibit SARM1 *independent* of its ability to increase NAD^+ levels.[7] Finally, although it can be difficult to compare mouse and human dosing, the equivalent NAM dose in humans would far exceed the lethal concentration. Thus, it may be that other ways of modulating the pathway, either alone or

in combination, may be more viable therapeutic options. Nonetheless, these results serve as the first proof-of-principle that modulation of NAD^+-dependent signaling can protect RGC structure and function in the setting of elevated IOP.

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Response by Pete A. Williams & Simon W. M. John on behalf of all authors

We thank Dr. Weinreb and the IGR editorial board for selecting our work for discussion. We are grateful to all of the reviewers, each a respected clinician scientist, for their time and thoughtful comments. This works extends literature on mitochondria in glaucoma by showing that mitochondrial dysfunction is an early abnormality within retinal ganglion cells in vivo in an inherited, ocular hypertensive glaucoma model. Furthermore, this work shows that levels of NAD or its precursor nicotinamide impact vulnerability to glaucoma. The growing literature linking mitochondrial susceptibility to glaucoma significantly raises excitement for the possibility that our findings will have importance for human glaucoma. Neuroprotective strategies against glaucoma are of great therapeutic need. However, we fully accept that, despite representing a large body of work, our paper is an early step with limitations. We agree that further animal studies and ultimately human trials are needed to assess the safety and efficacy of nicotinamide in glaucoma, and to allow firm conclusions about the general relevance of our findings to human glaucoma. We hope that our paper will serve as a stimulus for such studies.

To elucidate both the intraocular pressure- (IOP) and age- dependent mechanisms that drive retinal ganglion cell vulnerability in glaucoma, we utilized the DBA/2J (D2) mouse model of glaucoma. Our views on the appropriateness of this model for our studies and on some other comments respectfully differ to those from Dr. Quigley and are discussed in further detail below. We had attempted to give adequate methodology for all of the approaches in the paper with supporting references providing more details. The D2 mouse is arguably one of the most well characterized models of glaucoma. Although the greater damage in the anterior segment of D2 eyes (including the mild subclinical inflammation) must be considered and is important in the aetiology of IOP elevation, we are **not aware of any robust reported evidence indicating that the mechanisms of neural degeneration are very different in D2 mice compared to other glaucomas**. It remains possible that common pathways of neurodegeneration are induced by harmfully high IOP with the relative importance of specific processes varying between individual patients.

Importantly, the D2 model has various other features that are similar to human glaucoma including the variability of the IOP and neural phenotypes[1, 2], the topographic pattern of retinal ganglion cell loss[3], and the location of a critical insult to retinal ganglion cell axons in the optic nerve head.[3] Various pathways and molecules change in both D2 mice and in human glaucoma. Changes in the complement pathway, endothelin pathway, and mitochondrial dysfunction are all reported in human patients and in the D2 model.[1,4-11] The progressive variable nature of the IOP phenotype in D2 mice more closely resembles chronic human glaucomas than the suddenly induced IOP elevation in experimentally induced models. As IOP profiles and the timing between harmful IOP and retinal ganglion cell degeneration are also variable in the human disease, traumatically insulting the eye with lasers or microbeads is not necessarily better than the natural D2 disease. However, we fully agree that all of these models are complementary and that studies in a variety of models are needed. Induced models have potential to provide valuable experimental control for further studies, though in our hands we have found substantial variability in both the induction of IOP following the administered insult and the neurodegeneration in these models too.

Regarding elevated IOP, D2 mice are responsive to IOP lowering treatments that lessen the neurodegeneration (without changing the iris disease). Thus, the neural dysfunction and neurodegeneration in D2 eyes appears IOP-dependent.[12-16] Importantly, the genes conferring the anterior segment disease have been transferred to another mouse strain. The resultant mice develop an anterior segment disease that is indistinguishable in timing and severity to that of DBA/2J mice, but they do not develop high IOP or glaucoma. This demonstrates that the anterior segment disease does not cause glaucoma by itself [17, 18]. As mice of this second background develop glaucoma when IOP is elevated by other means (inherited or experimentally induced [19, 20]), these collective data strongly argue for a dominant role of high IOP in D2 glaucoma.

Dr Quigley states that we did not test age as a variable. We used RNA-sequencing of RNA from retinal ganglion cells to elucidate early mechanisms that underlie retinal ganglion cell vulnerability in glaucoma. We used samples from D2 and no glaucoma control mice (D2-*Gpnmb*$^{+}$) at two ages to elucidate both age- and IOP-dependant transcriptomic changes. Age was a key variable in this genomic study. Guided by our RNA-sequencing analysis we discovered an age-dependant decline in NAD(t) (NAD total; NAD^{+} and NADH). We used nicotinamide (an NAD precursor) treatment to restore NAD(t) levels and prevent

glaucoma in appropriately aged animals. Decreased NAD levels are increasingly becoming implicated in a broad array of aging deficits and age-related diseases.[21-24] Supported by this rapidly growing literature on NAD in aging and metabolic health, the decreased NAD levels in aged mice were hypothesized to increase vulnerability to mitochondrial dysfunction and glaucoma following periods of elevated IOP. We agree that we did not assess young and old animals with the same degree of IOP exposure. This is a complementary approach that controls for IOP exposure but does not invalidate our approach. Additionally, we agree that we did not test the efficacy of nicotinamide treatment in both young and old animals, in part because the decline of NAD(t) has not occurred in the young mice. This would be an approach to assessing the efficacy at both young and older ages (a different issue to the one that we addressed). Instead, we set out to therapeutically target the age-related decrease of NAD(t) levels in animals at ages when the glaucoma develops and this robustly protected these older mice. Due to its multiple effects, NAM may well protect at younger ages too, but such a result would not indicate that NAD(t) changes are unimportant for the age-related disease. Many groups studying aging use approaches that are similar to ours, and we prefer to use a natural disease model. In the future, it will be valuable to apply similar transcriptomic approaches to panels of improved, human-relevant, mouse models having human mutations in various human glaucoma genes (humanized models of glaucoma).

Regarding Dr. Quigley's methodological comments in relation to our use of the term disease progression (first comment). In this context, we had defined progression as based on progressive transcriptomic changes. The goal of these experiments was to identify gene expression changes that occur during disease initiation and very early molecular progression. This required the study of eyes that are not yet distinguishable by conventional optic nerve or retinal ganglion cell analyses, as such early molecular changes occur prior to neurodegeneration. To accomplish this, we used unsupervised hierarchical clustering (to identify molecularly defined groups based on transcript expression). The study design required closely age-matched mice and used a large number of samples that were individually analyzed by RNA-sequencing (initially 72 samples, plus an additional 10 samples from nicotinamide treated mice). This strategy identified 4 molecularly distinct groups or stages of early progression. These groups were ordered in terms of their increasing molecular distance from the age-, sex- and genetically-matched no glaucoma controls that were housed in the same environment. With this increasing distance from controls, the number of differentially expressed genes increased. Over the past decade, similar hierarchical clustering designs have transformed understanding of molecular processes contributing to a variety of biological processes and diseases. For example, they have provided key information about new molecular subtypes of cancer.[25,26] In the near future, such powerful genomic approaches will likely be adopted to improve clinical trials by allowing molecular stratification of disease types as well as stratification by molecular responses to treatments. There is ample evidence that such approaches identify groups with biologically meaningful molecular differences. We have previously used this hierarchical clustering approach to characterize and order early, pre-degenerative stages of glaucoma.[4,27] In the first of these studies, we included no glaucoma controls as well as mice at different stages of glaucoma (including pre-degenerative stages and stages with moderate or severe degeneration as determined by the degree of retinal ganglion cell axon and soma loss). Importantly, the unsupervised computational process sorted all of these samples into meaningful groups as evidenced by the fact that previously known groups consisting of

no glaucoma controls, moderate or severe glaucoma were successfully recreated using the unsupervised process.[4] This unsupervised but meaningful grouping of samples is clearly evident in our subsequent studies.[11,27] The recreation of biologically meaningful groups by the unsupervised hierarchical clustering, along with data that targeting the molecular processes identified through this grouping strongly protect from glaucoma[4,6,10,11], validates our approach for grouping samples as a valuable way to provide new mechanistic knowledge about glaucoma.

To target declining NAD(t) we chose the precursor nicotinamide (the amide of vitamin B3). Nicotinamide effectively raises NAD(t) levels in other systems, and was potent at raising NAD(t) levels in D2 retinas (by ~3-fold). For these experiments, we used 2 doses of nicotinamide, 550 mg/kg/d and 2000 mg/kg/d. In response to Dr. Pasquale's comments, an equal number of male and female mice were used for the drug studies, with sexes balanced in both treatments and control groups. We have not assessed lower doses. The human dose equivalents of our doses are 2.7 g/d and 9.8 g/d respectively for a 60 kg human.[28] Doses of 3-9 g/d of nicotinamide or nicotinic acid have been used long-term in humans for other conditions with few adverse effects (in some cases for up to 5 years). Doses of 3 g/d and below are generally considered to be safe. There are some cases of individual susceptibility to hepatotoxicity (reported on higher doses *e.g.* 9 g/d) but they are reversible and rare. Overall nicotinamide has a good risk to benefit ratio and has fewer unpleasant side effects than nicotinic acid. (For a review on nicotinamide safety see[29]) To follow up further on Dr. Pasquale's points, macular edema is a rare complication of nicotinic acid treatment (0.67% patients treated for hyperlipidemia) and is reversed by stopping treatment.[30] In the literature, the term niacin initially referred to nicotinic acid but sometimes refers to a mix of nicotinic acid and nicotinamide. It is unknown whether nicotinamide itself causes macular edema. Given the differing biochemical properties of these molecules including differences in their side effects, we propose the further testing of nicotinamide in glaucoma.

Nicotinamide treatment was profoundly protective in D2 mice. It also protected against TNFα-induced retinal ganglion death *in vivo* as well as against retinal ganglion cell death in an axotomy culture system. Regarding Dr. Quigley's comments on our optic nerve and retinal ganglion cell analyses in D2 mice, axon counts have been performed confirming the protection in treated mice. These data were not shown in the original *Science* paper but have now been included in a subsequent manuscript that is under consideration. We included data for our morphological determination of the degree of optic nerve damage using PPD stained nerves in the *Science* paper (Figure 2B). We included this data, as a large number of eyes were assessed using this approach, and as this technique has been validated against axon counting in various publications with high reproducibility between masked investigators, *e.g.*[3,4] PPD stains the myelin sheathes of axons darkly but more lightly stains other nerve components in healthy nerves. Its great value and sensitivity is in detecting individual stressed or damaged axons among a sea of healthy axons. It allows this by differentially staining the axoplasm of such stressed or damaged axons more darkly than that of healthy axons. It detects stress/damage in axons that otherwise appear healthy and have an intact myelin sheath. As the severity of injury to an axon increases it becomes very darkly stained. Since axon number is variable in mouse nerves, and since PPD allows sensitive detection of damaged axons, we argue that this is a very sensitive approach for detecting disease especially at early stages of disease. Importantly, our method not only considers the number of healthy axons (light staining axoplasm) and damaged axons (darkly staining

axoplasm) but also the cross sectional area of the nerve and the degree of gliosis. In the discussed study, >50% of the untreated control mice had very obvious and severe damage with massive axon loss and profound gliosis while this phenotype was only present in 3% of mice treated with the higher dose of nicotinamide. Dr. Quigley discusses numbers of eyes that need to be compared and that we only counted retinal ganglion cells for 8 eyes in each experimental group (shown in Figure 2C). We only counted retinal ganglion cells in 8 eyes per group, as this was only to confirm that the protection of retinal ganglion cell axons and retinal ganglion cell bodies were not uncoupled as distinct molecular processes participate in the degeneration of these neuronal compartments[31,32] (*i.e.* that cell bodies were saved in eyes with protected axons but lost in eyes with axon loss). Our optic nerve analysis (Figure 2B) shows the robust nature of the nicotinamide protection. Figure 2B contained data for >50 eyes per group. The sample size is listed in the legend for Figure 2B, panel F as the nerve images are shown in F. The same data is plotted as a histogram in Figure 2B. We apologize that this may not have been adequately clear. In total, Figure 2B shows data for 191 nicotinamide treated nerves and 136 untreated controls. Adding confidence of the importance of NAD increasing treatments for glaucoma, the protection has proven robust for >620 treated DBA/2J eyes that we have now published. (Breakdown: 274, nicotinamide treatment alone; 349, *Wld*^{S} gene, *Nmnat1* gene therapy, or combination of gene therapy and nicotinamide (see below); 315, untreated controls[3,7,10,11]). Regarding axon transport, we used cholera toxin B with a fluorescent tag as a marker of axoplasmic transport. Although not empirically quantified, labeling was absent from all brains with severe optic nerve disease while there was qualitatively normal cholera toxin B labeling in the superior colliculus and lateral geniculate nucleus of protected mice with no optic nerve disease. Combined with pattern electroretinography analysis this demonstrates that in nicotinamide treated mice retinal ganglion cells and their axons are both histologically and functionally protected from glaucoma.

We next tested a gene therapy, over-expressing *Nmnat1* (a terminal enzyme in NAD production). NMNATs has been shown to be protective in other axon degeneration systems and in induced glaucomas.[21,33-35] Gene therapy of *Nmnat1* was protective against glaucoma in D2 eyes and showed additional protection when combined with nicotinamide administration (at the lower dose of 550 mg/kg/d). We agree with Dr. Martin's comment on a somal/nuclear versus axonal site of action for this treatment. In this gene therapy experiment, the entire gene of interest (*Nmnat1*) was overexpressed. The nuclear localization signal (NLS) was intact, and so the protein product (NMNAT1) should be localized predominantly at the nucleus within the soma. Poly ADP ribose polymerase (PARP) increases in retinal ganglion cells during pre-degenerative stages of D2 glaucoma. Since PARP depletes nuclear NAD and the NMNAT1 NLS was intact, it seems most **likely that NAD-mediated protection is mediated within the nucleus of the cell body; however, a role within axons cannot be ruled out.**

Dr. Welsbie raises an important point regarding potential independent effects of nicotinamide and NMNAT1. Given the roles of NAD in aging and metabolism, increasing NAD(t) levels are likely to be a key component of the protection. In this context NMNATs are likely to be the main effector. We have previously demonstrated that WLD^{S} can protect D2 eyes from glaucoma.[3] In a recent publication, we demonstrated that WLD^{S} increases NAD(t) levels in the retina, and that the **combination of WLD^{S} plus nicotinamide offers near-complete protection from glaucoma** (94% eyes have no detectable glaucoma).[10]

This further supports a key role for increased NAD(t) levels, but other NAD independent effects of WLD^S cannot be ruled out as at least partially contributing to the protection. Dr. Welsbie also points out that NMNAT1 has an inhibitory effect on SARM - an important NAD degrading molecule during axon degeneration. In addition to being an NAD precursor, nicotinamide also has other importance molecular properties that might contribute to its protective effect in glaucoma including inhibition of the NAD consumers CD38[36], PARPs[37], and histone deacetylases[23,38], as well as ADP-ribosyl cylase[39-41] which impacts vascular tone and calcium signalling. This combination of functions may explain why nicotinamide is so protective against D2 glaucoma.

To conclude, we thank the commentators for their thoughtful discussion and insightful comments. We look forward to working with the glaucoma research community to continue exploring the role of mitochondrial biology, NAD, and NAD-based treatments in glaucoma.

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Meeting Highlights

Top-four of the 12th Congress of the Spanish Glaucoma Society

Valencia, Spain, March 23–25, 2017

Francisco J. Muñoz-Negrete

Anja Tuulonen, Tampere, Finland

There is no evidence for an optimum test set in the diagnosis and follow-up of glaucoma. Different technologies give variable results with poor agreement. Risk of bias in the published study designs is significant. Sensitivity and specificity values indicate a large variability. Long-term follow up could serve as the most appropriate reference standard.

Gema Rebolleda, Madrid, Spain

A significant widening and deepening of the optic disk cup, Bruch´s membrane opening enlargement and prelaminar tissue thinning occurred following intravitreal aflibercept injection (IAI) for neovascular age macular degeneration. Eyes showing greater morphological changes associated with a lower IOP increase post-injection, suggesting that optic nerve head compliance might buffer the effect of IAI on IOP values.

Arranz-Marquez E., Madrid, Spain

One hundred healthy, young myopic eyes were divided in two groups by their CCT (matched for age, and refractive error). The group with the thinnest CCT presented a significantly thinner OCT-RNFL. Thin CCT is a well-known risk factor for glaucoma development. Our finding that a thin CCT is associated with thinner RNFL suggests a connection between both parameters.

Miguel Tudela-Molino, Murcia, Spain

Ninety-nine glaucoma patients and 58 controls performed the Pittsburgh, Berlin and Epworth questionnaires, and standard automated perimetry and OCT (macular and peripapillary scans). Glaucomatous patients had poorer results on the Pittsburgh and Berlin questionnaires. Both groups showed worse perimetric results with higher sleepiness. Finally, higher risk of OSAS was related to reduced inner retinal thicknesses in the glaucomatous group.

Top-six of the Glaucoma Group—Association of Ophthalmologists of Latvia Annual Meeting

Riga, Latvia, March 9–12, 2017

Guna Laganovska

Analysis of genetic polymorphisms in operated primary open-angle glaucoma patients in the Latvian population

Kristine Baumane, Riga

A possible association of several single nucleotide polymorphisms (SNPs) with POAG was determined in the Latvian population. In total, three SNPs were genotyped in all samples: rs4656461 near the TMCO1 gene, rs1063192 near the CDKN2B gene and rs10483727 near SIX1/SIX6 gene. A small difference in the allele frequency of rs4656461 among different age groups was observed.

Oskars Gertners, Riga

Anterior segment optical cocherence tomography is a useful tool which can be used to assess the ultrastructure of a filtering bleb after trabeculectomy. Postoperative intraocular pressure values can be linked with a few indicators in filtering bleb ultrastructure which show the overall outcome after trabeculectomy surgery.

Jekaterina Varlamova, Riga

Accurate and precise intraocular pressure measurement is essential in detection and management of glaucoma. Reducing IOP has been shown to lessen progressive loss of the visual field. Goldmann applanation tonometer is regarded as the ‘gold standard’ however alternative methods have reduced risk of the possible cross infection.

Dāvis Raščevskis, Riga

Thin cornea can be a marker of a structural weakness in the back of the eye – at the optic nerve level. This might mean a greater likelihood of the damage occurring in the optic nerve and suggests that lower intraocular pressure is required.

Ēriks Elksnis, Gulbene

The tear osmolarity is an important indicator of the ocular surface health. Long-term anti-glaucomatous eye drop use is accompanied not just by the discomfort symptoms of the ocular surface, but also with increased tear film osmolarity.

Ilze Lace, Riga

Secondary glaucoma is one of the most common complications after silicone oil injection in vitreoretinal surgeries. In order to achieve good IOP control and prevent further IOP increase, early disease management and timely silicone oil removal is essential.

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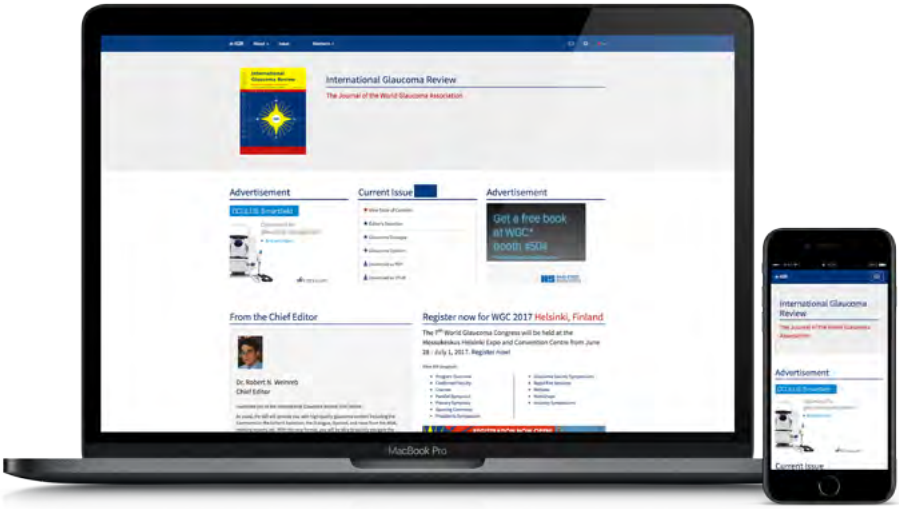

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Editor's Selection

With the multitude and variety of publications it seems almost impossible for the ophthalmologist to intelligently read all the relevant subspecialty literature. Even the dedicated glaucomatologist may have difficulty to absorb 1200+ yearly publications concerning his/her favorite subject. An approach to this confusing situation may be a critical selection and review of the world literature.

Robert N. Weinreb, Chief Editor

Quality of Life

Which surgery?

Comment by David Friedman, Baltimore, MD, USA

71426 Quality of life in the Tube Versus Trabeculectomy Study; Kotecha A, Feuer WJ, Barton K, Gedde SJ,American Journal of Ophthalmology 2017; 176: 228-235

Kotecha and colleagues have published the five-year quality of life (QoL) outcomes of the Tube Versus Trabeculectomy Study which compared tube shunt to trabeculectomy in eyes with previous ocular surgery. Two hundred twelve patients aged 18-85 years of age enrolled at 17 clinical centers were randomized to either have tube shunt or trabeculectomy surgery to lower intraocular pressure. The 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25) was administered at baseline and then annually, mostly over the phone.

Over five years, 13% of the cohort died and 18% more were lost to follow-up. Of those remaining, only 51% completed NEI VFQ at five years. Those who completed the five-year survey had better QoL scores at baseline indicating a possible bias. Furthermore, visual field data were only available for the operated eye, which could have been the better or worse eye, a limitation because most QoL surveys show that self-reported QoL is most strongly related to the better eye visual field.

The authors defined a 'Minimal important Difference' (MID) as two dB of VF loss and ten letters on ETDRS. As seen in other publications, MID change in VA had a greater impact on the NEI-VFQ score than does change in MD. Loss of VA or MD in the better eye resulted in a declined in the NEI-VFQ score. That said, overall there was almost no change overall in NEI-VFQ score over course of study. The authors did not report specifically on those who experienced complications such as hypotony or diplopia after surgery.

In summary, this study supports previous research findings and also found that **among those still answering the QoL questions at five years, no differences existed between those who had trabeculectomy and those who had tube shunt surgery**.

Anatomical Structures

RNFL thinning rate topography

Comment by Andrew Tatham, Edinburgh, UK

71410 Trend-based progression analysis for examination of the topography of rates of retinal nerve fiber layer thinning in glaucoma; Lin C, Mak H, Yu M, Leung CK; JAMA ophthalmology 2017; 135(3): 189-195

Trend-based progression analyses can be used to quantify rates of change in visual field or structural measurements in glaucoma, with perimetry and imaging devices incorporating software for this purpose. By performing linear regression of measurements over time it is possible to obtain annualized rates of change and estimate the risk of future visual disability.

By examining rates of RNFL change throughout the OCT scan it may be possible to improve accuracy of progression detection

Progression analysis using optical coherence tomography (OCT) has largely relied on measurements of the circumpapillary retinal nerve fiber layer (cpRNFL) and several studies have shown faster rates of cpRNFL loss to be associated with increased risk of worsening visual field loss.[1,2] Routinely performed OCT scanning protocols often capture information about a much larger area of RNFL than the cpRNFL and the premise of Lin and colleagues' work was that restricting progression analysis to evaluation of cpRNFL alone may reduce the ability to detect localized changes and to detect progression outside the

circumpapillary region. In other words, by examining rates of RNFL change throughout the OCT scan it may be possible to improve accuracy of progression detection and perhaps better predict visual field changes.

A 'rates of change RNFL thickness map' was developed and used to examine the topography of rates of RNFL thinning. The authors used the Cirrus OCT (Carl Zeiss Meditec) to obtain a 6 x 6 mm^2 optic disc region RNFL thickness map and examined rates of RNFL change in individual 50 x 50 superpixel regions. The initial study examined rates of change in RNFL thickness in 240 eyes of 139 patients with POAG over a follow-up period of at least five years.[3] One hundred seventeen eyes were found to have progressive RNFL loss using the RNFL thickness change map, which was associated with an eight-fold increased risk of worsening visual field loss during follow-up.[3]

The current study examined the topography of RNFL thinning in the 117 eyes progressing on the RNFL thickness change map and scrutinized the mean and peak rates and the location of faster rates of loss. During the follow up period, a median of 18 OCT scans was acquired for each eye. The rates of change RNFL thickness map showed wide variation in rates of RNFL thinning between eyes and at different locations, with faster loss observed in the inferotemporal and superotemporal regions, particularly closer to the optic disc margin. **Faster mean and peak rates of RNFL thinning were significantly associated with visual field progression**, even after accounting for confounding factors such as age, IOP, CCT, axial length and average cpRNFL thickness at baseline. Over a follow-up period of 4.9 to 7.2 years, 23% and 24% of eyes had visual field progression defined by event-based analysis (based on Early Manifest Glaucoma Trial (EMGT) criteria) and pointwise trend analysis respectively. Each 1 µm/year faster rate of mean RNFL loss was associated with a 39% increased risk of visual field progression by the EMGT criteria and 18% by pointwise trend analysis. The rates of change RNFL thickness map seemed to perform well even in eyes with moderate to advanced glaucoma at baseline, with faster rates of change remaining indicative of visual field progression. **However, the area of progressive RNFL thinning was not indicative of visual field worsening.**

Further work is needed, particularly to compare the ability of measures of rates of change in cpRNFL and change in RNFL thickness map to predict visual field loss, and to examine whether adjustment for age-related RNFL loss will improve performance. However, the rates of change RNFL thickness map shows promise as a tool for assessing progression as it maps the rate and location of RNFL loss and provides further information compared to assessments based solely on cpRNFL. This could be particularly important given the variation in rates of change in RNFL over time at different locations between and within eyes.

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Anatomical Structures

Visualizing ONH structures in OCT images

Comment by Michele Iester, Genova, Italy

71463 A digital staining algorithm for optical coherence tomography images of the optic nerve head; Mari JM, Aung T, Cheng CY, Strouthidis NG, Girard MJ; Translational Vision Science & Technology 2017; 6: 8

Jean-Martial Mari *et al.* have reported the results of a new method to analyse optic nerve head (ONH) and highlight either connective or neural tissue by using spectral domain optical coherence tomography (OCT). This new algorithm was verified with a digital phantom, compared with a modern clustering algorithm, and tested in 10 subjects with consistent digital stains.

It has been shown that in healthy ONHs the proportion of connective and neural tissue can change on the basis of the position: in the anterior part neural tissue is about 90%, while 10% in the posterior part. Connective tissues are the main load bearing elements of the ONH, and there is evidence to suggest that biomechanical and/or morphologic features of these tissues may serve as strong biomarkers for glaucoma, as the authors mentioned in their work.

There is evidence to suggest that biomechanical and/or morphologic features of these tissues may serve as strong biomarkers for glaucoma

In previous studies, different authors have shown structural changes, such as collagen types, which can change from a long chain to a short one in glaucomatous patients, direction of the collagen fibres, or change in elastin production, which could alter the biomechanical proprieties of the connective tissue and in particular of the lamina cribrosa (LC). Besides in previous studies it has been shown that LC changes morphologically and morphometrically its shape in glaucomatous patients, even after a surgical approach the LC tends to invert its shape for IOP changes. In a recent study, Yang *et al.* summarized that the main connective tissue changes associated with chronic IOP elevation in a monkey model were post-laminar deformation, laminar thickening, scleral canal expansion, laminar migration, and scleral bowing, outlining the importance of monitoring connective tissue behaviour *in vivo*. Thus, because ONH connective and neural tissues are altered in glaucoma, digital staining might be of interest in the clinical management of glaucoma.

Using OCT images of the ONH, **they found that this new algorithm could be able to isolate connective tissues, prelaminar tissues and the nerve fibre layer, or other retinal layers, as four separate digitally stained volumes, and that connective tissues of the ONH were highly visible in the digitally stained images.**

We spent many years before having retinal segmentation programs, but now we start to have new data to improve our clinical approach. Automated segmentation of the LC and of the choroidal vessels has remained a challenge, and only few solutions, sometimes complex, have been proposed. However this is beyond the scope of this paper, but in future a simple segmentation algorithms could be combined with digital staining to automatically identify structures such as the anterior LC surface or the choroidal vessels.
This is a prelaminar study and there are some points that need to be considered such as the small number of included patients, the type of included patients and the absence of correlation with the human histology for lack of available data. Besides there are some technical limitation that the authors mention in discussion, but they will work on in the next future. This study is another step forward in the analysis of the deeper ocular structures.

Anatomical Structures

Under pressure: deformation of the lamina cribrosa

Comment by Crawford Downs, Birmingham, AL, USA

71321 The pressure-induced deformation response of the human lamina cribrosa: Analysis of regional variations; Midgett DE, Pease ME, Jefferys JL, Patel M, Franck C, Quigley HA, Nguyen TD; Acta biomaterialia 2017; 53: 123-139

Optic nerve head (ONH) biomechanics has been hypothesized to play an important role in the development and progression of glaucoma, but it is not well understood. The dearth of available data is due to the technical challenges involved in the measurement of ONH tissue mechanical properties (stiffness) and the complexity of the ONH and scleral geometry. Further complicating the study of ONH biomechanics is the biologic variability in the load-bearing structure, which includes geometry (scleral thickness, neural canal shape and size, laminar pore size and beam thickness, etc.), and tissue stiffness, which may change with age, pathology, extracellular matrix (ECM) composition, and connective tissue remodeling.

Midgett, Nguyen and coworkers quantified the pressure-induced deformation of the lamina cribrosa in eight human eyes from six donors using second harmonic image generation (SHG) microscopy. Mechanical strain was estimated using digital volume correlation analysis of the image volumes taken at pressures from five to 45 mmHg. **Results suggest that older age was associated with lower strain, indicating a stiffer lamina with age**, although this result is limited by the small sample size (n = 6) and cross-sectional nature of the study, and so must be confirmed. **Sectoral analysis also suggested that laminar**

strains were highest in the temporal and inferior quadrants, which is important as these regions are most associated with focal glaucoma damage. Finally, tensile strains in the plane of the sclera/lamina were much higher than the shear and compressive strains, which is important when one considers the possible mechanisms of biomechanical damage to the axons in the laminar region.

In-plane tension is the dominant strain component in the lamina

As the authors acknowledge, these results should be viewed with caution due to the small sample size, and the assumptions and approximations inherent to the strain calculation methods. In addition, SHG imaging requires imaging the lamina from the posterior side, necessitating dissecting away the retrolaminar optic nerve to expose the laminar structure. This is difficult, and some of the laminar structure may have been cut away in this process, thereby altering its mechanical response. In addition, experimental evidence in dogs and pigs indicates that retrolaminar tissue pressure in the optic nerve never falls below ~4 mmHg due to pial tension, which contributes to the translaminar pressure difference *in vivo*. Removal of the optic nerve for imaging also removes this back pressure, which is likely to alter the measured laminar biomechanics somewhat. In spite of these limitations, this paper is important in providing evidence that in-plane tension is the dominant strain component in the lamina.

Fortunately, recent advances in OCT and other imaging technologies are improving, which may lead to more comprehensive clinical assessments of ONH biomechanical behavior in the near future.

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Basic science

The aqueous humor lipidome in POAG

Comment by Sanjoy Bhattacharya, Miami, FL, USA

71534 Changes in the lipidomic profile of aqueous humor in open-angle glaucoma; Cabrerizo J, Urcola JA, Vecino E; Journal of Glaucoma 2017; 26(4): 349-355

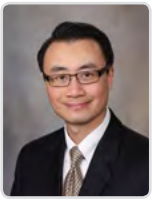
Cabrerizo *et al.* conducted a comparative profiling study of the aqueous humor lipidome from open-angle glaucoma (OAG) patients and control subjects (n = 10 samples per group). Their findings are in agreement with previously published reports on phospholipid, sphingolipid and cholesterol metabolite changes both in human glaucoma and a hypertensive mouse (DBA/2J) model. Particularly**, they found several sphingomyelin and cholesteryl ester species to be upregulated in glaucomatous aqueous humor**. Authors speculate that observed patterns of lipid metabolism may reflect increased synthesis of sphingomyelins by sphingomyelin synthase or conversely – due to decreased sphingomyelinase activity. They also point out that the increase in sphingomyelin, cholesterol and phosphocholine levels may be implicated in oxidative stress metabolic response in glaucoma.

The work of Cabrerizo *et al.* adds up to a complex picture of lipid dysregulation arising in glaucoma as well as providing another data resource for new scientific ideas to emerge. The next challenges lie in understanding biological significance of metabolic alterations associated with glaucoma and possibly translating this knowledge into clinical application.

There are some potential limitations to the study. They utilized ultrahigh performance liquid chromatography with time of flight (TOF) mass spectrometry. Different types of mass spectrometry combined with derivatization and prior separation by asymmetric field ion mobility produces superior and higher confidence results than a singular mass spectrometric method alone. An example is the same sample being analyzed by both triple quadrupole and Orbitrap high-resolution mass spectrometers. TOF mass spectrometers necessitate frequent calibration due to inherent drift in the tube. However, while NMR methods are now reliable for targeted metabolomics, they do not handle mixtures of lipids such as the one offered by aqueous humor samples well. As well, the bioinformatic softwares and databases for lipidome analyses have advanced, but still some challenges remain. Despite the limitations, the metabolomics and lipidomic studies are coming along. The details from these studies will eventually complement the proteomic and genomic information and help provide a further detailed picture of the metabolic changes in glaucoma.

Animal Models

Pulsatile aqueous outflow in primates



Comment by Arthur Sit, Rochester, MN, USA

71574 Aqueous angiography in living nonhuman primates shows segmental, pulsatile, and dynamic angiographic aqueous humor outflow; Huang AS, Li M, Yang D, Wang H, Wang N, Weinreb RN; Ophthalmology 2017; 124(6): 793-803

Aqueous humor outflow is typically modelled as a steady-state system using the modified Goldmann equation, which describes IOP as a function of aqueous humor production rate, outflow facility, and episcleral venous pressure and uveoscleral outflow rate. However, previous investigators have demonstrated a pulsatile nature to aqueous humor outflow, which may be problematic for the Goldmann model.[1] Huang *et al.* investigated this issue in a study to examine the dynamic nature of aqueous humor outflow in the aqueous and episcleral veins.

As with cadaver eyes, flow was found to be segmental with regions of flow and no-flow.

Using a novel technique that they have called aqueous angiography**, the authors infused fluorescein or indocyanine green (ICG) into the anterior chamber of non-human primates** (NHP). Fluorescent images of the outflow tract were used to capture and document the presence of aqueous humor flow. Concurrent OCT imaging was used to match the angiography images to the structural location of aqueous and episcleral veins.

As with cadaver eyes, flow was found to be segmental with regions of flow and no-flow

This technique has previously been used by this group on cadaver eyes,[2] and was adapted to living NHP eyes for this study. **As with cadaver eyes, flow was found to be segmental with regions of flow and no-flow. However, the authors also reported pulsatile flow, and dynamic shifts where flow would stop in one region and begin in another**. These results demonstrate a previously undocumented dynamic and variable nature to distal outflow.

The authors also reported pulsatile flow, and dynamic shifts where flow would stop in one region and begin in another

While the results of this study are very interesting, it was limited by the short duration (three to nine seconds) of the videos obtained. Therefore, dynamic changes could be observed, but it is unknown if these were short transient changes or longer term shifts in flow patterns. As well, since the entire eye could not be imaged in a single video, it is not known if the overall flow rate remained constant during the shifts in pattern. Nevertheless, aqueous angiography appears to be a novel technique for investigating the physiology of the distal aqueous humor outflow pathway. The use of tracers compatible with living eyes makes use of the technique in humans a possibility. **Adaptation of this technique to human patients may enable better understanding of the effect of glaucoma therapies, and improved targeting of devices that alter outflow patterns, such as minimally invasive glaucoma surgeries.**

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Clinical Examination Methods

Automated kinetic perimetry

Comment by Chris Johnson, Iowa City, IA ,USA

71424 Reclaiming the periphery: Automated kinetic perimetry for measuring peripheral visual fields in patients with glaucoma; Mönter VM, Crabb DP, Artes PH; Investigative Ophthalmology and Visual Science 2017; 58: 868-875

In this paper, the authors assess the relationship between visual field sensitivity for the central 30 degrees radius of the visual field (static perimetry) and the far periphery (kinetic perimetry beyond 30 degrees) in a group of 30 glaucoma patients using the procedures on the Octopus 900 perimeter. Evaluation of the visual field beyond 30 degrees in glaucoma patients was a research topic of interest in the 1970s and 1980s, but there has been little effort directed towards this problem in recent times. As a consequence, the advances in visual field testing have not been applied to assessment of the far periphery. **The authors demonstrate that it is possible to have a reliable and efficient method of testing the far periphery using automated kinetic testing.** Additionally, they provide examples where there is good agreement between central and peripheral visual fields, as well as cases in which the central visual field is damaged but the peripheral visual field is intact, and vice versa. Because the peripheral visual field is important for navigation, object

detection and motion sensitivity, it is refreshing to see this this issue being investigated again. The results of testing the far periphery will provide important information to the practitioner concerning the patient's capabilities for performing activities of daily living and the influence of damage to these visual field areas on quality of life. Although this study concentrated on glaucoma patients, it is clear that quantitative assessment of the far peripheral visual field will be even more important for patients with retinal degenerations or neuro-ophthalmologic problems affecting the visual pathways. **Development and refinement of methods to evaluate the far periphery will undoubtedly enhance the practitioner's ability to predict which activities will be affected by impairments the visual system.** I applaud the authors for their efforts on this problem.

Clinical Examination Methods

Detecting perimetric changes

Comment by Boel Bengtsson and Anders Heijl, Malmö, Sweden

71364 Detecting change using standard global perimetric indices in glaucoma; Gardiner SK, Demirel S; American Journal of Ophthalmology 2017; 176: 148-156

In this study, Gardiner & Demirel compared regression analyses of three standard perimetric summary indices to determine which index can detect statistically significant deterioration earliest in eyes without severe cataract and with POAG 'and/or likelihood of developing glaucomatous damage'. Most eyes must have lacked manifest glaucoma based on most recent MD values. **On the average MD showed deterioration somewhat sooner (7.3 years) than VFI (8.5 years), while PSD changes appeared later (10.5 years)**. In what the authors call 'moderately damaged eyes, with most recent MD values between -0.505 (!) and -19.5 dB', the worst half of their material, MD and VFI did not differ significantly, but 'were almost equivalent'. This must mean that in eyes with field loss MD and VFI must have been very similar.

MD and PSD were rather insensitive for detection of progression events

Detecting glaucoma progression events based upon the significance of regression slopes of global indices, as the authors have done was standard in the 1980's. It soon became apparent, however, that MD and PSD were rather insensitive for detection of progression events, as reported by Chauhan, Drance & Douglas in 1990.1

These early experiences led to the development of analyses focusing on localized change, *e.g.*, glaucoma change probability maps and Progressor. These and other types of event analysis were used in the important CRTs, *e.g.*, AGIS, CIGTS, EMGT and recently UKGTS, and performed well.

Today, linear regression of VFI and MD is used to determine rates of progression, rather than progression events. **A statistically significant and very shallow slope is usually without clinical importance. In contrast a steep, but still statistically non-significant slope may require immediate attention.** We commend the authors for confirming these historical findings.

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Clinical Examination Methods

How High-Definition can you go?

Comment by Brad Fortune, Portland, OR, USA

71225 Imaging individual neurons in the retinal ganglion cell layer of the living eye; Rossi EA, Granger CE, Sharma R, Yang Q, Saito K, Schwarz C, Walters S, Nozato K, Zhang J, Kawakami T, Fischer W, Latchney LR, Hunter JJ, Chung MM, Williams DR; Proceedings of the National Academy of Sciences of the United States of America 2017; 114: 586-591

A landmark paper reporting the first successful imaging of retinal ganglion cells in the living human eye

Ethan Rossi, with a team of investigators led by David Williams at the University of Rochester Medical Center, published earlier this year a landmark paper reporting the first successful imaging of retinal ganglion cells in the living human eye.[1] This is a major milestone with significant relevance to glaucoma research. Because neuronal cell bodies within the retina produce very low backscatter, they are nearly transparent and extremely

difficult to detect – even by advanced ocular imaging modalities such as adaptive optics scanning light ophthalmoscopy (AOSLO). Rossi *et al.* overcame this challenge by modifying the AOSLO configuration to have an off-axis detection scheme, in which light is collected from portions of the image plane adjacent to the confocal position. The theoretical explanation for the consequential benefit, as first proposed by Elsner, Burns and colleagues,[2,3] is that light passing through the confocal aperture along the optical axis represents predominantly backscatter, which masks the fainter contrast produced by multiply scattered light. However, areas of the image plane away from the optical axis contain relatively more light that has undergone multiple scatter within the retina – and thus, additional information. Previous studies cited by Rossi *et al.* had shown that off-axis detection methods could be used to enhance contrast of otherwise faint retinal structures (*e.g.*, cells of the outer retina and blood vessel walls), but none had successfully detected neurons of the inner retina. Rossi *et al.* in their study used an offset aperture approach and systematically evaluated the effects of offset distance, offset direction and aperture size as well as various combinations of images from different aperture positions to enhance contrast of retinal cells, including those within the ganglion cell layer. **The structures they observed within the retinal ganglion cell layer using the 'multi-offset' approach matched closely the size of retinal ganglion cells known from post mortem histopathology studies**. **The investigators provided further evidence by imaging anesthetized macaque monkeys simultaneously with the multi-offset AOSLO and a two-photon technique designed to detect intrinsic fluorescence from ganglion cells.** Also, the higher light levels used for multi-offset AOSLO imaging in the monkey eye enabled the investigators to detect even subcellular structure in some of the putative retinal ganglion cells.

While these exciting results offer a view with potentially transformative impact for glaucoma research, numerous hurdles remain to be addressed before this remarkable achievement enjoys wide spread implementation. For example, the authors pointed out that the specific combination of offset aperture positions producing the best contrast enhancement in the ganglion cell layer varied in unpredictable ways between imaging locations, as well as within the field of view (1.2° to ~1.5° square) of each imaging area. This study was also limited to retinal locations where the overlying nerve fiber bundles were thin, sparse and ganglion cell bodies were spread in a single layer such as along the temporal raphe, in a small number of eyes. More work is needed to optimize the array of offset positions and image combinations, as well as to image ganglion cells stacked several layers thick nearer to the fovea.

Numerous hurdles remain to be addressed before this remarkable achievement enjoys wide spread implementation

Remarkably, there are already indications that these challenges are being met by other means. Indeed, it was exhilarating to see the results presented during ARVO 2017 by Zhoulin Liu and a group of investigators led by Don Miller at Indiana University,[4] who were also able to image retinal ganglion cells in the living human and quantify their diameter and density in three dimensions using AO-OCT running at an A-line acquisition rate of 500

KHz. Using this approach, Liu and colleagues were able to image ganglion cells even at locations where they were stacked four to five rows deep or beneath thicker nerve fiber bundles.

Collectively, the recent report by Rossi *et al*. and the emerging results of Liu *et al.* point to a bright future when direct imaging of retinal ganglion cells will be possible in a clinical setting.

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Clinical Examination Methods

An OCT-A perspective on peripapillary capillaries and POAG

Comment by Tae-Woo Kim, Bundang-gu, Seongnam, Korea

71399 Peripapillary perfused capillary density in primary open-angle glaucoma across disease stage: an optical coherence tomography angiography study; Geyman LS, Garg RA, Suwan Y, Trivedi V, Krawitz BD, Mo S, Pinhas A, Tantraworasin A, Chui TY, Ritch R, Rosen RB; British Journal of Ophthalmology 2017 Feb 1 (E-pub ahead of print)

With the emergence of optical coherence tomography angiography (OCTA), it became possible to image the papillary and peripapillary microvasculature in a non-invasive manner. It has been demonstrated that the peripapillary capillary density is decreased in eyes with glaucoma compared to healthy eyes. In addition, the decrease capillary density has been shown to coincide with the retinal nerve fiber layer defect identified by red-free photography. These observations raise the possibility that the peripapillary vessel density may be a viable modality for monitoring POAG. Geyman *et al.* assessed the peripapillary capillary density (PCD) in 60 POAG eyes with varying stages of disease and 24 healthy control eyes. The PCD was calculated as a percentage as the ratio of pixels associated with perfused capillaries to the total number of pixels in the corresponding region of interest

(ROI). A custom automated algorithm was used to isolate the capillary microvasculature. **They found a progressive stepwise decrease of PCD from control eyes throughout worsening POAG stage.** PCD demonstrated a comparable diagnostic capability to the cpRNFL thickness and visual field parameters. In addition, PCD exhibited significant correlations with cpRNFL thickness and VF MD.

An intriguing finding of this study is that the **temporal peripapillary sector exhibited a far smaller decrease across POAG stage relative to other sectors.** The less decrease was contrasted with circumpapillary retinal nerve fiber layer thickness (cpRNFLT), particularly in late stage of disease: while the cpRNFLT demonstrated a marked thinning in the temporal sector, the temporal PCD was relatively preserved. This finding raises a possibility that the PCD may have better correlation with VF than cpRNFLT in the end stage of disease. The RNFLT may reach to its 'floor' despite remaining VF. Therefore, serial measurement of the RNFLT is often not useful to monitor disease progression in eyes with advanced VF defect. It would be of interest to see whether the PCD progressively attenuates along with the VF loss throughout the whole stage of disease. If so, the PCD may help clinicians to monitor the disease progression by enabling them to look at the vasculature-function consistent worsening of disease until the end stage of disease

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Refractive Errors and Glaucoma

Optic disc tilt in yopia


Comment by Sunee Chansangpetch and Shan Lin, CA, USA

71023 Patterns of damage in young myopic glaucomatous-appearing patients with different optic disc tilt direction; Lee JE, Lee J, Lee JY, Kook MS; Journal of Glaucoma 2017; 26: 144-152

One of the leading hypotheses on glaucomatous damage in myopic eyes is related to the mechanical strain on optic nerve axons. Optic disc tilt is a typical characteristic found in myopic eyes. This tilt feature may cause a mechanical distortion of the lamina cribosa leading to axonal damage.[1]

Lee and colleagues hypothesized that different patterns of disc tilt may differentially affect the pattern of damage. Their study compared patterns of 'glaucomatous-appearing' damage between vertical disc tilt (VDT) and horizontal disc tilt (HDT) in young myopic eyes. They enrolled 52 eyes for each group with age, degree of refractive error, and severity of neural rim loss matching.

> HDT is an independent risk factor for having more advanced glaucomatous-appearing visual field defects

The study found that HDT had significantly greater chance for nasal step and Bjerrum visual field defect subtypes compared to VDT which was more likely to have paracentral and temporal wedge defects. **The frequency of glaucomatous-appearing visual field defects was significantly higher in HDT compared to VDT**. The authors concluded that HDT is an independent risk factor for having more advanced glaucomatous-appearing visual field defects (MD < -6).

Although it was not described in the study that the RNFL defect location from optic disc photography correlated with the location of the visual field defects, the study had the strength of using objective criteria in determining the tilt characteristic and masking all disc and visual field grading.

Of note, the mean peak outpatient IOP was approximately 17 mmHg, indicating that a majority of the subjects were normal-tension type. This observation may suggest the presence of mechanical susceptibility of myopic eyes to glaucoma at any given IOP level, at least in a Korean population.[2] It also should be emphasized here, as the authors acknowledge, that the subjects are those who had a mild degree of visual field defect (MD < -10) or no visual field defect at all. Thus, the patterns of glaucomatous-appearing damage in this

study may be present in glaucoma patients as well as non-glaucomatous myopic patients. Therefore, it is still inconclusive, given the cross-sectional nature of the study, whether these visual field defects are the result of glaucomatous damage or represent pre-existing, non-progressive findings in myopic eyes.

In conclusion, the information and analysis from this paper provides another aspect for clinicians to consider in myopic patients – that certain optic nerve tilt directions may contribute to higher chance of myopia-related visual field defects which could also be pattern-specific. Furthermore, there is a possibility that these defects show greater susceptibility for subsequent glaucomatous damage.

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Clinical Forms of Glaucoma

Corneal biomechanics in NPG

Comment by Cynthia Roberts, Columbus, OH, USA

70916 Corneal biomechanical parameters and asymmetric visual field damage in patients with untreated normal tension glaucoma; Li BB, Cai Y, Pan YZ, Li M, Qiao RH, Fang Y, Tian T; Chinese Medical Journal 2017; 130: 334-339

The authors are to be congratulated for a well-designed study to investigate the potential contribution of biomechanics to glaucomatous damage in asymmetric normal-tension glaucoma (NTG). Two important features of the design include that subjects were newly diagnosed, so the results are not influenced by treatments which may affect biomechanical response, and that intraocular pressure (IOP) was not different between fellow eyes. **Higher IOP leads to a stiffer response simply due to the nonlinear properties of the cornea, and not necessarily the presence or absence of disease.**[1] The device used was the Corvis ST, which produces a large number of dynamic corneal response (DCR) parameters which are extracted from 140 images captured during a 30 ms air puff induced deformation of the cornea.[2]

The authors have correctly interpreted the differences in DCR's between the worse eye and the better eye. However, it is recommended to use more standard biomechanical terminology. Stiffness defines resistance to deformation. Greater resistance is a stiffer eye, and

lower resistance is a more compliant eye, or a softer eye. Therefore, where the authors conclude that the shape of the cornea is more easily changeable in the worse eye, it is recommended to instead state that **the worse eye was more compliant or softer than the better eye, in the presence of similar IOP and central corneal thickness**.

The corneal biomechanical deformation response is influenced not only by the properties of the cornea, but also the properties of the sclera due to displaced fluid when the cornea becomes concave

It is important to note that the corneal biomechanical deformation response is influenced not only by the properties of the cornea, but also the properties of the sclera due to displaced fluid when the cornea becomes concave. Stiffer boundary conditions limit corneal deformation, which leads to the conclusion that a stiffer sclera will be associated with stiffer corneal behavior.[3] Therefore, it is difficult to determine whether the cornea, or the sclera, or both are softer in the worse eye. In addition, it is not yet known how these biomechanical differences influence the development of NTG. However, the current study is an important contribution to this field. A novel stiffness parameter for glaucoma will be reported at the 2017 World Glaucoma Conference.[4]

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Medical Treatment

Properties of generic latanoprost solutions

Comment by Louis Cantor, Indianapolis ID, USA

71551 The physical properties of generic latanoprost ophthalmic solutions are not identical; Kolko M, Koch Jensen P; Acta Ophthalmologica 2017 Feb 22 (E-pub ahead of print)

The authors are to be congratulated for investigating an important clinical topic. While generic formulations exist in order to reduce cost and increase access to therapy, generic ophthalmic medications may pose a number of challenges, which may be especially problematic when treating a chronic ocular disease. The authors highlight a number of variations in formulation and physical properties of several generic latanoprost products when compared to the reference legend drug, or brand product. **Within the US there are at least eight generic formulations for latanoprost available.** In addition, an individual patient may receive a different generic formulation when obtaining refills, further compounding these issues.

> Despite these reported variations between generic formulations, the majority of patients appear to still respond as well as to the branded product, but clinically there are notable exceptions, in terms of efficacy or tolerability

While the US FDA and other international organizations have guidance which is intended to ensure that generic and branded formations are equivalent, differences continue to be reported. Despite these reported variations between generic formulations, the majority of patients appear to still respond as well as to the branded product, but clinically there are notable exceptions, in terms of efficacy or tolerability. In addition, in a cost-effectiveness model, if 'costs' related to poor compliance, disease progression, additional office visits and other factors are considered, the actual cost of the drug may not be the key factor driving costs long term. Therefore, as a matter of practice, I recommend that a patient be scheduled for a follow-up visit within one to two months any time we make a switch from a branded to generic formulation.

I recommend that a patient be scheduled for a follow-up visit within one to two months any time we make a switch from a branded to generic formulation.

In addition, I instruct patients to contact our office to schedule an earlier appointment any time they obtain a refill with a different looking bottle or label, as this suggests their pharmacy has just switched them to a different generic product. As physicians, our challenge is to ensure that each patient is receiving their optimal individual therapy.

Medical Treatment

Round-the-clock effects of IOP lowering eyedrops

Comment by Franz Grehn, Wurzburg, Germany

71499 Efficacy and safety of preoperative IOP reduction using a preservative-free fixed combination of dorzolamide/timolol eye drops versus oral acetazolamide and dexamethasone eye drops and assessment of the clinical outcome of trabeculectomy in glaucoma; Lorenz K, Wasielica-Poslednik J, Bell K, Renieri G, Keicher A, Ruckes C, Pfeiffer N, Thieme H; PLoS ONE 2017; 12: e0171636

The major long-term challenge of filtration surgery is excessive wound healing and failure by scar formation of the new outflow route. The wound healing process is significantly influenced by the amount of conjunctival inflammation resulting from long-term antiglaucomatous topical medication. Most topical antiglaucoma medications and preservatives induce inflammation. **Preoperative reduction of inflammation is a pivotal step to better postoperative bleb development.** Therefore, many surgeons use regimens to quiet inflammation preoperatively.

> In a subgroup of severe preoperative inflammation from medication, the addition of preoperative steroids may be still be considered

The present study compares two different preoperative regimens, (1) dorzolamide/timolol preservative free combination versus (2) acetazolamide/dexamethasone as pretreatment four weeks prior to surgery after discontinuation of their individual preoperative topical medication. The two arms of this randomized prospective study included 30 and 32 patients, respectively. IOP decrease after surgery at three months follow-up was used as the primary outcome measure. Secondary outcomes were the number of 5-FU injection needed, needlings, suture lysis, hypotension rate, visual acuity, and bleb morphology. The adverse events were studied with the vision-related NEI VFQ25 test.

Regarding IOP change no differences or inferiority could be found with the dorzolamide/timolol preservative free regimen at three months (IOP decrease -8.12 mmHg versus -8.30 mmHg) and at six months (9.13 mmHg versus -9.06 mmHg). All secondary parameters were also not significantly different. The NEI VFQ 25 test battery showed increased reporting of AE's in the azectazolamide/dexamethasone group.

The results demonstrate that reducing the preoperative medication to either a fixed combination of unpreserved dorzolamide/timolol or treating preoperative IOP with oral acetazolamide plus topical steroids is equally effective. However, the role of preoperative topical steroids for suppression of inflammation should be further investigated as result might also depend on the severity of preoperative inflammation which was not quantified in this study. Although the initial inflammation was balanced by randomization, in a subgroup of severe preoperative inflammation from medication, the addition of preoperative steroids may be still be considered and their usefulness should be tested in a subsequent study as proposed by the authors.

Clinical Forms of Glaucoma

Blood pressure dips and ONH hemorrage in NPG

Comment by Ki Ho Park, Seoul, South Korea

71290 Association between nocturnal blood pressure dips and optic disc hemorrhage in patients with normal-tension glaucoma; Kwon J, Lee J, Choi J, Jeong D, Kook MS; American Journal of Ophthalmology 2017; 176: 87-101

There is still debate on the pathogenic mechanism of optic disc hemorrhage (ODH), specifically on the question of whether mechanical or vascular factors predominate. The current study by Kwon *et al.* supports the hypothesis that ODH is a manifestation of vascular dysregulation in patients with normal-tension glaucoma (NTG). It is the strength of their study that it enrolled a large number of consecutive 349 NTG patients (698 eyes) who were ≥ 40 years old and had undergone 24-hour intraocular pressure and ambulatory blood pressure (BP) monitoring in the habitual position. The patients were classified into two groups: 'non-physiologic' dippers, including nondippers and overdippers, and 'physiologic' dippers. Nocturnal BP overdipping was defined as nighttime BP reduction > 20%, as calculated by the following formula: [(daytime mean MAP – nighttime mean MAP)/daytime mean MAP] X 100. Nighttime BP overdippers showed a significantly greater frequency of ODH than did nondippers or dippers. **Being an overdipper was a significant as well as an independent risk factor for ODH occurrence during follow-up**.

Additionally, in a multivariate analysis, the standard deviation of daytime MAP was shown to be one of the risk factors associated with ODH, which means that **MAP fluctuation is associated with ODH.** Further, daytime MAP was higher in overdippers (95.1 mmHg) than in physiologic dippers (92.7 mmHg) or nondippers (87.5 mmHg). In other words, the nighttime BP overdippers in this study were also daytime BP overpeakers. For example, if a patient had a daytime MAP of 150 and a nighttime MAP of 100, the nighttime BP reduction would be (150-100/150) x 100 = 33%. And for the same patient, the daytime BP elevation could be calculated, by the same formula, as (150-100/100) x 100 = 50%.

There is a possibility that ODH is associated not only with nighttime BP reduction but also with daytime BP elevation

So, there is a possibility that ODH is associated not only with nighttime BP reduction but also with daytime BP elevation. Given that individuals on antihypertensive agents were not excluded from the study, there remains the possibility that patients with systemic hypertension were enrolled, over-treated, and, thus showed low nighttime BP subsequently. It should be noted, in this respect, that systemic hypertension has been reported as an ODH risk factor among Korean NTG patients.[1]

The current study supports an IOP-independent mechanism of ODH in some proportion of NTG eyes; however, it should be emphasized that in order **to understand the pathogenesis of ODH, the systemic vascular mechanism cannot be considered independently of localized structural vascular susceptibility and the pressure balance among IOP, cerebrospinal fluid pressure and arterial/venous pressure around the optic nerve head**.[2]

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1. Kim YD, Han SB, Park KH, *et al.* Risk factors associated with optic disc haemorrhage in patients with normal tension glaucoma. Eye (Lond) 2010;24(4):567-572.
2. Suh MH, Park KH. Pathogenesis and clinical implications of optic disk hemorrhage in glaucoma. Surv Ophthalmol 2014;59(1):19-29.

Medical Treatment

Round-the-clock effects of SLT and travoprost

Comment by Norbert Pfeiffer, Mainz, Germany

71469 The effects of selective laser trabeculoplasty and travoprost on circadian intraocular pressure fluctuations: A randomized clinical trial; Kiddee W, Atthavuttisilp S; Medicine 2017; 96: e6047

This paper compares the efficacy of IOP reduction by either travoprost or selective laser trabeculoplasty in terms of mean IOP reduction and IOP fluctuation over a 24 hour period in 58 eyes that were randomized to either treatment. In brief, both travoprost and SLT treatment lowered IOP significantly by -4.1 and -3.7 mmHg, respectively with no statistically significant difference between both treatments.

> Travoprost effect seemed to be present during day and night, while the SLT effect was significant only during nighttime

IOP fluctuations were < 3 mmHg over a 24 hour diurnal measurement period in 100% of travoprost **and 87% of SLT eyes**. Percentages were similar for eyes with normal tension glaucoma (96% and 82%, respectively). However, the travoprost effect seemed to be present during day and night, while the SLT effect was significant only during nighttime. Travoprost effect seemed to be present during day and night, while the SLT effect was significant only during nighttime

This study addresses the important question whether patients may be better controlled on medical of laser therapy. The strengths of the study include a randomization with comparable groups, a 24 hour IOP measurement before and after treatment and a clear definition of success. However, the sample size was small (16/14 eyes for POAG and 14/16 eyes for NTG. Masking could have been better with sham laser treatment and placebo given to the laser group. Also, an untreated control group what help to understand a possible effect of regression to the mean which might account for at least some of the effects shown here. Yet, the very popular use of SLT warrants studies like these to better understand the efficacy of laser treatment.

Surgical Treatment

What does SLT do to Schlemm?

Comment by Ronald Fellman, Forth Worth, Texas, USA

71263 Microarchitecture of Schlemm Canal before and after selective laser trabeculoplasty in enhanced depth imaging optical coherence tomography; Skaat A, Rosman MS, Chien JL, Ghassibi MP, Liebmann JM, Ritch R, Park SC; Journal of Glaucoma 2017; 26(4): 361-366

The authors utilized optical coherence tomography (OCT) with enhanced depth imaging (EDI) with the OCT anterior segment module to visualize the microarchitecture of Schlemm's canal before and four weeks after selective laser trabeculoplasty (SLT). Thirteen primary open-angle glaucoma eyes, average age 68 years, were included for analysis. Both 180 and 360-degree treatments were utilized, but the proportion is unknown. Intraocular pressure decreased on average 5 mmHg. The majority of the eyes were phakic.

The authors found **the nasal cross-sectional area (CSA) of Schlemm's canal increased by 8% after SLT and was correlated with IOP drop along with an increase in the volume of the canal**.

If technically feasible, a recommendation would be to include OCT of the temporal portion of the angle as well. For the patients that only underwent nasal SLT, the temporal angle OCT microarchitecture could be important from a non-treated viewpoint. For example, if the authors found an increase in cross-sectional area of the nasal canal but not temporally, this would imply the increase in canal area was mainly the result of increased aqueous flow in the region induced directly by the SLT as opposed to an increase in canal diameter due simply to a lower IOP. This would better delineate the mechanism of how SLT lowers IOP in glaucoma patients.

Clearly, a great deal of information can be learned about the canal and its surroundings with non-invasive OCT. With similar methodology in a study of the CSA of Schlemm's canal measured by OCT, these same authors found a 24% increase in the CSA of the canal after the instillation of pilocarpine 2%. As the technology improves, our delineation of the anatomy and physiology of the outflow system will ultimately lead to better canal-based surgical outcomes. Glaucoma surgeons and outflow researchers eagerly await these technologically challenging imaging advancements.

Surgical Treatment

What does SLT do to Schlemm?

Comment by Murray Johnstone, Seattle, WA, USA

71263 Microarchitecture of Schlemm canal before and after selective laser trabeculoplasty in enhanced depth imaging optical coherence tomography; Skaat A, Rosman MS, Chien JL, Ghassibi MP, Liebmann JM, Ritch R, Park SC; Journal of Glaucoma 2017; 26(4): 361-366

This study uses OCT to explore the effects of SLT on Schlemm's canal (SC) dimensions in 13 eyes. Imaging was done with commercially available SD-OCT image acquisition and 3D volumetric analysis software. **Mean IOP was significantly reduced 4 weeks after OCT; (From 19.8 ± 7.6 to 14.4 ± 3.8 mm Hg; P = 0.003). Mean SC cross-sectional area (CSA) increased by 8% reaching statistical significance: (P = 0.029)** and SC CSA was significantly positively correlated with post-SLT SC expansion (P = 0.023, R = 0.622).

The authors use the above results as a warrant to conclude that SLT results in SC expansion in eyes of patients with POAG. The authors point out that SC imaging by OCT is challenging because of tissue depth, shadowing by superficial vessels and intrinsic structural variations in SC dimensions. Other limitations involve inclusion of only the nasal quadrant of the eyes which may not be representative of the entire SC circumference.

In view of SC anatomic variability even small changes in positioning of the OCT beam before and after laser Rx may result in sampling quite different regions. Additional limitations include the need for manually delineating both the hyporeflective SC lumen and alignment of B-scans. It is reassuring that despite the above limitations significant SC CSA changes could be identified.

Physical expansion of SC is suggested by the authors to be directly related to increased aqueous outflow and IOP reduction. They conclude that *in vivo* OCT imaging techniques may be useful to evaluate the action of laser procedures, pharmacologic agents, surgical techniques, and devices. While the current study requires laborious techniques that may not be clinically practical, the study does a great service in pointing to a future likely to have further improvements in imaging acquisition and automated analysis algorithms. Such evaluative capabilities may well provide major advances in glaucoma management.

Surgical Treatment

How does SLT work and on whom?

Comment by John Samples, Portland OR, USA

71656 Mechanism of action of selective laser trabeculoplasty and predictors of response; Gulati V, Fan S, Gardner BJ, Havens SJ, Schaaf MT, Neely DG, Toris CB; Investigative Ophthalmology and Visual Science 2017; 58: 1462-1468

This study details the first thorough investigation of aqueous humor dynamics in the common clinical procedure selective laser trabeculoplasty (SLT). Numerous papers have reported on argon laser trabeculoplasty (ALT), often attributing its effects on outflow pathway extracellular matrix to stretching or the release of cytokines, but the other types of trabeculoplasty such as ALT and micropulse laser trabeculoplasty (MDLT) have been reported on much less frequently. SLT was introduced in the late 1990s and has been increasingly popular among clinicians in the last decade, sometimes being used as first-line therapy particularly in patients who do not wish to use medications or who are likely to be non-compliant with the use of medication. SLT treatment delivers about 1% of the total energy to the trabecular meshwork compared to the amount delivered by an ALT. Yet, SLT is similar to ALT in its clinical efficacy. This raises substantial questions about its mechanisms. SLT has purportedly has different mechanisms of action from ALT. Histological analysis has shown that SLT applications in the meshwork lack the cellular destructive and coagulative effects found in the meshwork with ALT.

Prior to this study, conventional outflow facility had been reported to increase three months after SLT treatment alone with no effects on aqueous humor inflow. Other parameters of aqueous humor dynamics which can alter intraocular pressure (IOP) include episcleral venous pressure (EVP) and uveoscleral outflow. This is the first study to look at all of the aqueous humor dynamic properties in the same patients. Thirty-one patients were enrolled and 29 completed the study. None of the subjects were on any pressure lowering medications. They were either interested in SLT as first line therapy, were poor at compliance or wanted to stop current medications due to side effect or cost concerns; all of which are frequently encountered clinical situations that can make laser procedures desirable. It is interesting that 31 out of 36 consecutively evaluated candidates for the study met these criteria. The completed data set consisted of 29 subjects with 29 study eyes and 25 contralateral eyes. The latter were particularly important because over the years, contralateral IOP effects have been attributed to trabeculoplasty treatments.

As expected, SLT showed a significant decrease in both 9AM and noon time pressure measurements in the SLT treated eye at three months compared with baseline. **The study did not find any statistically significant association between IOP response and demographics or treatment variables including total laser energy, angle pigmentation or**

percentage of laser spots with visible response. The association between young age and greater IOP response, which has previously been reported for ALT, was almost statistically significant. An interesting association with central corneal thickness also approached statistical significance but did not quite achieve it, suggesting that an additional 1.0 mmHg of pressure lowering response was associated with 25 um thinner cornea.

Methodologically, two methods were used in this study, fluorophotometry and tonography. A decrease in uveoscleral outflow after SLT was seen using the fluorophotometric method but not the tonographic method. Conceivably, as the authors speculate, a small decrease in uveoscleral outflow may reflect quantitatively that more aqueous is transiting the trabecular meshwork.

This study shows that three months after SLT IOP reduction is mediated through an increase in outflow facility**.** No meaningful effects of any other parameters such as episcleral venous pressure or uveoscleral outflow were found. No contralateral eye effects were found. **Both a higher baseline aqueous inflow and a lower baseline outflow facility were found to be predictive of IOP response to SLT.**

It might be that we will be able to predict which patients will best respond to the trabeculoplasties when we have the ability to identify who has a high baseline aqueous flow and a lower baseline outflow facility. It is intriguing that in a larger study or in a study with different glaucoma subpopulations thin cornea may be found to be associated with a better SLT response, but that did not achieve statistical significance here.

This meaningful and well-done paper really encompasses the methods that we glaucomatologists need to use to evaluate not only drugs but our other new laser, ultrasound and minimally invasive surgical procedures. Understanding mechanisms will be enhanced, as this work points out, when we have better methods of measuring episcleral venous pressure and also uveoscleral outflow.

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Surgical Treatment

Preopertaive IOP reduction


Comment by Tomas Grippo and Facundo Sanchez, New Haven, CT, USA

71359 The 24-hour effects of brinzolamide/brimonidine fixed combination and timolol on intraocular pressure and ocular perfusion pressure; Seibold LK, DeWitt PE, Kroehl ME, Kahook MY; Journal of Ocular Pharmacology and Therapeutics 2017; 33(3): 161-169

Seibold *et al.* performed a prospective, randomized study comparing, over a 24-hour period, the effect on IOP, heart rate (HR), blood pressure (BP) and ocular perfusion pressure (OPP) of a fixed combination of brinzolamide 1% / brimonidine 0.2% (BBFC) versus timolol 0.5% drops, in patients with ocular hypertension or OAG. The main value of this study rests on the fact that it is the first one evaluating the effect of BBFC throughout a 24-hr period, together with the assessment of systemic parameters.

> We still do not fully understand the clinical impact of the lack of nocturnal hypotensive effect of some drops with concomitant alteration of other parameters like 24hr OPP on the glaucomatous process

The authors found that **BBFC TID and timolol BID, both lowered IOP during the diurnal period. However, only BBFC decreased IOP overnight**. **Timolol had little effect on BP, while BBFC caused a significant reduction particularly in systolic BP. Timolol also caused a greater decrease in HR**. BBFC had no effect on OPP, while timolol increased it only during the diurnal period. The authors propose that the overall lack of effect on OPP of BBFC is likely related to the reduction in systolic BP.

The study is well-designed and methodologically performed up to the high standards of the classic literature in the subject. It has a few limitations related to the design itself (open label), and other limitations we have still at present when evaluating IOP, like the impossibility of measuring it as a continuous variable.

Although great progress has lately been done, we still do not fully understand the clinical impact of the lack of nocturnal hypotensive effect of some drops with concomitant alteration of other parameters like 24hr OPP on the glaucomatous process. Despite that, Seibold *et al.*'s study adds to our understanding of what happens with these variables under the effect of BBFC throughout a 24-hr cycle, helping us build up the ground of knowledge necessary to make progress in that direction.

Surgical Treatment

Tube vs. tube

Comment by Paul Chew and Victor Koh, Singapore

71315 Five year pooled data analysis of the Ahmed Baerveldt comparison study and the Ahmed versus Baerveldt Study; Christakis PG, Zhang D, Budenz DL, Barton K, Tsai JC, Ahmed II; ABC-AVB Study Groups; American Journal of Ophthalmology 2017; 176: 118-126

The TVT study showed that the glaucoma drainage device (GDD) had higher success rates with lesser complications leading to the increasing trend of GDD implantation. The five-year pooled data analysis of the Ahmed Baerveldt Comparison Study and the Ahmed Versus Baerveldt Study is timely in providing information on the efficacy profile of the two most popular GDDs.

Similar to the TVT study, the eyes included in this study had advanced glaucoma and multiple risk factors for trabeculectomy failure. **The five-year results showed that the cumulative probability for failure (defined when intraocular pressure [IOP] > 18 mmHg, < 6 mmHg or less than 20% reduction) is significantly lower for Baerveldt compared to Ahmed group (37% vs. 49%, P = 0.01).** This relationship persisted even if the upper limit of IOP is set at 15 mmHg (48% vs. 61%, p = 0.002). However, this suggested that both types of implants are still less than desirable if a lower target IOP is required for patients with more advanced glaucoma. The most common reason for failure was high IOP for both implants but the Baerveldt group had significantly higher proportion of refractory hypotony. The Ahmed group are twice more likely to require additional de novo glaucoma surgery compared to the Baerveldt group (p = 0.01)

Both implants showed impressive reduction in mean IOP (49% reduction in Ahmed group and 58% reduction in Baerveldt group) and mean number of glaucoma medications (42% reduction in Ahmed group and 55% reduction in Baerveldt group).

Clinically, this pooled data analysis suggested that **the Baerveldt implant is more appropriate for eyes which require much lower target IOP including advanced glaucoma and young patients. On the other hand, Ahmed valve implant would be more suitable for eyes at risk of hypotony (such as inflammatory and neovascular glaucoma) and eyes which require urgent IOP lowering.**

Health Economics

Cost analysis of treating PACG by early lens extraction

Comment by Anja Tuulonen, Tampere, Finland

71288 Early lens extraction with intraocular lens implantation for the treatment of primary angle closure glaucoma: an economic evaluation based on data from the EAGLE trial; Javanbakht M, Azuara-Blanco A, Burr JM, Ramsay C, Cooper D, Cochran C, Norrie J, Scotland G; BMJ open 2017; 7: e013254

The landmark EAGLE study represents the first pragmatic glaucoma cost-effectiveness analysis based on three-year trial data. As the effects of treatment are expected to persist further into the future, in addition a Markov model was developed to extrapolate the results up to ten years (assumptions of some input parameters derived also the from literature).

> Lens extraction was estimated 67-89% on being cost-effective at three years

The data set consisted of 285 patients with primary angle closure glaucoma in 22 UK centers. Using web-based randomization, **145 eyes received early lens extraction and 140 laser peripheral iridotomy**. Effectiveness was measured in terms of quality-adjusted life years (QALY) gained by completing EQ-5D questionnaire at baseline, six, 12, 24 and 36 months. Glaucoma Utility Index was administered as disease-specific instrument. All cost elements were summed to generate a total cost per patient for 2012–2013 (UK perspective). The primary economic outcome was the incremental cost per QALY gained (Incremental Cost-Effectiveness Ratio, ICER).

The incremental cost for lens extraction was £ 981 for QALY gain of 0.069, yielding an ICER of £ 14 282 per QALY. Lens extraction was estimated 67-89% on being cost-effective at three years with ceiling willingness-to pay at £ 20 000.

The model based on extrapolation suggested that lens extraction may become a cost-saving strategy over ten-year horizon. Although the complete utility and cost data were not available in 37% of patients and the mean cost and utility values were assumed to be constant beyond three years, the deterministic sensitivity analysis showed that model-based findings were generally robust. Pragmatic randomized study design with adequate randomization, intention-to-treat analysis and collection trial based data for economic analysis are strengths of the study.

The EAGLE study encourages to design further pragmatic cost-effectiveness studies in order to confirm that every-day glaucoma care improves patients' well-being and is worth the money spent, both in diagnostics and treatment.

Miscellaneous

Are intensive workouts good for you?

Comment by Pradeep Ramulu, Baltimore, MD, USA

71480 The relation between exercise and glaucoma in a South Korean population-based sample; Lin SC, Wang SY, Pasquale LR, Singh K, Lin SC; PLoS ONE 2017; 12: e0171441

In this study, Lin and colleagues evaluated the association of self-reported physical activity and glaucoma, defined through optic nerve photos and a single FDT test, in a population-based study of Korean adults. **The authors found that men with glaucoma were more likely to report high levels of physical activity as compared to men without glaucoma**, while no similar relationship was present in women. Amongst men, but not women, the authors also found that the 3.3% of men reporting vigorous exercise seven days a week more likely to have glaucoma than a comparison group of men performing vigorous exercise three times a week. The findings complicate an already-muddy landscape regarding the impact of physical activity on glaucoma.

> The findings complicate an already-muddy landscape regarding the impact of physical activity on glaucoma

In mice, an emerging body of evidence suggests that activity can protect against the harmful effects of raised IOP. **In humans, most studies on the topic, including the current study, have evaluated the relationship between activity and glaucoma cross-sectionally, leaving it unclear if activity patterns preceded or followed the onset of glaucoma**. Also, while self-reported activity can serve as a marker of real-world activity, it can differ substantially from physical activity measured by objective measures, which has been demonstrated to be restricted in persons with visual field damage. Clearly, future longitudinal studies employing detailed visual testing and cutting-edge activity monitoring are required to better delineate the relationship between physical activity and glaucoma. Given the current level of evidence, one would be wise to suggest that their glaucoma patients continue to lead an active lifestyle. **While the effect of this activity on their glaucoma is unclear, a vast literature has documented broad positive effects of an activity lifestyle that should not be discounted easily.**

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NEWS FLASHES

- ★ As for many studies in animal models of glaucoma, a key consideration is how generalizable the results are to human glaucoma
- ★ Their results support the therapeutic use of NAM in glaucoma; yet, they used NAM doses that are probably not feasible in humans
- ★ First, NAM treatment may behave differently in DBA/2J mice and it should be tested in other glaucoma models …
- ★ Although it can be difficult to compare mouse and human dosing, the equivalent NAM dose in humans would far exceed the lethal concentration
- ★ MD and PSD were rather insensitive for detection of progression events
- ★ There is a possibility that ODH is associated not only with nighttime BP reduction but also with daytime BP elevation
- ★ As with cadaver eyes, flow was found to be segmental with regions of flow and no-flow
- ★ The authors also reported pulsatile flow, and dynamic shifts where flow would stop in one region and begin in another
- ★ Despite these reported variations between generic formulations, the majority of patients appear to still respond as well as to the branded product, but clinically there are notable exceptions, in terms of efficacy or tolerability.
- ★ In-plane tension is the dominant strain component in the lamina.
- ★ In a subgroup of severe preoperative inflammation from medication, the addition of preoperative steroids may be still be considered
- ★ Travoprost effect seemed to be present during day and night, while the SLT effect was significant only during nighttime
- ★ The findings complicate an already-muddy landscape regarding the impact of physical activity on glaucoma.
- ★ By examining rates of RNFL change throughout the OCT scan it may be possible to improve accuracy of progression detection
- ★ Lens extraction was estimated 67-89% on being cost-effective at three years
- ★ A landmark paper reporting the first successful imaging of retinal ganglion cells in the living human eye.
- ★ Numerous hurdles remain to be addressed before this remarkable achievement enjoys wide spread implementation.
- ★ We still do not fully understand the clinical impact of the lack of nocturnal hypotensive effect of some drops with concomitant alteration of other parameters like 24hr OPP on the glaucomatous process
- ★ HDT is an independent risk factor for having more advanced glaucomatous-appearing visual field defects
- ★ The corneal biomechanical deformation response is influenced not only by the properties of the cornea, but also the properties of the sclera due to displaced fluid when the cornea becomes concave.

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