Effect of Timolol on Aqueous Humor Outflow Facility in Healthy Human Eyes

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• PURPOSE: **Hyposecretion of aqueous humor has been postulated to adversely affect the health of the trabecular meshwork and outflow resistance. However, the effect of medications that reduce aqueous humor production on outflow facility in living human eyes is unclear. This study evaluated the effect of timolol, an aqueous humor flow suppressant, on outflow facility in healthy eyes.**

• DESIGN: **Prospective, before-and-after study.**

• METHODS: **In a multicenter study, 113 healthy participants over 40 years of age were included. Intraocular pressure (IOP) was measured with the participant in the sitting position by using a pneumatonometer. The outflow facility was measured with the participant in the supine position by 2-minute pneumatonography. After participants self-administered drops of timolol 0.5% for 1 week, twice daily in each eye, both measurements were repeated.**

• RESULTS: **Mean IOP decreased from 15.1 ± 3.0 mm Hg at baseline to 12.4 ± 2.4 mm Hg ($P < 0.001$) after 1 week of timolol use. Mean outflow facility decreased from 0.23 ± 0.08 μL/min/mm Hg at baseline to 0.18 ± 0.08 μL/min/mm Hg ($P < 0.001$) after timolol. The change in outflow facility was negatively correlated with baseline outflow facility ($r = -0.51$; $P < 0.001$).**

• CONCLUSIONS: **Timolol reduces outflow facility in healthy human eyes, and this effect is greater in eyes with higher baseline outflow facility. This phenomenon may be related to reduced aqueous humor flow, but the precise mechanism remains to be determined. (Am J Ophthalmol 2019;202:126–132. © 2019 Elsevier Inc. All rights reserved.)**

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GLAUCOMA IS A PROGRESSIVE OPTIC NEUROPATHY and is the second most common cause of blindness in the world, with more than 2.5 million people affected in the United States alone.[1] Elevated intraocular pressure (IOP) is the primary risk factor in glaucoma and lowering IOP is currently the only effective treatment. IOP can be lowered in several ways including reduction of aqueous humor production or improvement of aqueous humor outflow facility.

Timolol is a nonselective beta-adrenergic antagonist[2] that has been widely used for glaucoma treatment since the late 1970s. It lowers IOP in healthy volunteers[3–5] and in patients with open-angle glaucoma[6–15] or ocular hypertension[12,14,15] by suppressing aqueous humor production 33% to 50% as measured by fluorophotometry.[4,5,16–21]

The effect of aqueous humor flow suppression on other parameters of aqueous humor dynamics, including aqueous humor outflow facility, is not well understood. Previous investigators have suggested that suppression of aqueous production could cause underperfusion of the trabecular meshwork, leading to a reduction in outflow facility.[22,23] However, several studies in patients with ocular hypertension or glaucoma have reported that timolol had no significant effect on outflow facility measured by tonography[11–13,19,24–30] or fluorophotometry,[30] whereas some studies have even reported an increase in outflow facility.[20,31,32] In those previous studies, all outflow facilities were measured in patients with dysfunctional trabecular meshwork, as indicated by a low outflow facility at baseline. The effect of aqueous humor suppression on outflow facility in healthy human eyes (with healthy trabecular meshwork) has not been reported. This study evaluated the effect of timolol on outflow facility in healthy human subjects.

METHODS

THIS PROSPECTIVE BEFORE-AND-AFTER STUDY WAS approved by the Institutional Review Boards at Mayo Clinic, University of Michigan, and University of Nebraska Medical Center. The study followed the tenets of the Declaration of Helsinki and was carried out in accordance with the regulations in Health Insurance Portability and Accountability Act of 1996. All subjects provided written informed consent to participate after the nature and possible risks of the study were discussed with them.

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• **STUDY SUBJECTS:** A total of 113 healthy participants, 40 years of age and older were enrolled from local area residents, employees, and patients of the ophthalmology departments at Mayo Clinic Rochester, University of Michigan, and University of Nebraska as part of a multicenter study (Glaucoma Biomarkers; NCT01677507). Each participant underwent a general health interview and comprehensive ophthalmologic examination including visual acuity, IOP by pneumatonometry, gonioscopy, slit-lamp biomicroscopy, A-scan biometry, ultrasound pachymetry, and fundoscopy. Subjects were excluded if they had a history or evidence of intraocular surgery, laser treatment, ocular pathology (including narrow angles or any form of glaucoma), ocular trauma within the previous 6 months, current use of any IOP-lowering medication, serious hypersensitivity to any components of the study medications, recent changes to systemic medications that might affect IOP, use of any glucocorticoid by any route, were known to be pregnant, and any contraindication for treatment with the study glaucoma medications including severe reactive airways disease and bradycardia.

• **MEASUREMENTS:** *Intraocular pressure.* Baseline IOP was measured in both eyes of each subject with the subject in the sitting position by using a pneumatonometer (Model 30 Classic, Reichert Inc., Buffalo, New York). The subject was then placed in a supine position and IOP was remeasured after 5 minutes. All IOP measurements were made between 9 and 11 AM. Calibration of the tonometer was verified according to the manufacturer's instructions, and the tip was cleaned before each set of measurements was made. Topical proparacaine 0.5% was instilled before each IOP and tonography measurement. The right eye was always measured first.

Outflow facility by pneumatonography. Outflow facility was measured with the subject in the supine position by using a pneumatonometer with a 2-minute tonography option. Outflow facility was calculated from the pressure decay curve digitized from paper tracing and using Langham's pressure-volume relationship tables and a polynomial fitted to the decay curve, as described previously.[33,34] The right eye was always measured before the left eye.

Axial length and central corneal thickness. Axial length was measured by A-scan biometry and central corneal thickness was measured by ultrasound pachymetry.

• **STUDY MEDICATIONS:** Intraocular pressure and outflow facility were measured at baseline prior to any medication use. Subjects were then instructed to instill timolol 0.5% solution, 1 drop every 12 hours in both eyes. The drug was self-administered for 1 week. After 1 week of treatment, IOP and outflow facility were remeasured by using the same techniques as used for the baseline measurements.

TABLE 1. Subject Characteristics

Characteristic	
Number of men	27
Number of women	86
Age (y)	
Mean ± SD	55.3 ± 8.9
Range	40–81
Race	
White	98
Black	11
Other	4
CCT (μm)	551 ± 39
Axial length (mm)	23.93 ± 1.23

CCT = Central corneal thickness; SD = Standard deviation.

• **STATISTICAL ANALYSIS:** Significance of changes in IOP and outflow facility from baseline in response to timolol were determined by using generalized estimating equation models to account for possible correlation between fellow eyes of the same subject (R Core Team 2018, RStudio version 1.1.456 software, GEE version 4.13-19; Vienna, Austria). Correlation between baseline outflow facility and changes in outflow facility after timolol treatment was determined by linear regression analysis. Differences were considered significant if P was less than 0.05.

RESULTS

A TOTAL OF 113 HEALTHY PARTICIPANTS (27 MALES AND 86 females; 98 whites, 11 African Americans, and 4 subjects of other races), 40 to 81 years of age (55.3 ± 8.9 years) were included in the study (Table 1). Among these subjects, 200 eyes from 104 subjects had satisfactory 2-minute IOP recordings that were suitable for calculation of tonographic outflow facility. The other 26 eyes did not have outflow facility tracings of adequate quality for outflow facility calculation.

The mean IOP at baseline was 15.1 ± 3.0 mm Hg (n = 200 eyes) and decreased to 12.4 ± 2.4 mm Hg ($P < 0.001$) after 1 week of timolol therapy (Table 2, Figure 1). The mean outflow facility at baseline was 0.23 ± 0.08 μL/min/mm Hg and decreased after 1 week of timolol treatment to 0.18 ± 0.08 μL/min/mm Hg ($P < 0.001$) (Figure 2), a mean change of 24.5%. In a subgroup analysis, the mean outflow facility decreased after 1 week of timolol treatment compared to baseline at each study site, from 0.22 ± 0.08 to 0.16 ± 0.07 μL/min/mm Hg at the University of Michigan ($P < 0.001$); from 0.23 ± 0.07 to 0.20 ± 0.07 μL/min/mm Hg at the Mayo Clinic ($P = 0.01$); and

TABLE 2. Effect of Timolol on Intraocular Pressure and Outflow Facility

	Baseline	Timolol	
	Mean ± SD	Mean ± SD	*P*
IOP (mm Hg)	15.1 ± 3.0	12.4 ± 2.4	<0.001
Outflow facility (μL/min/mm Hg)	0.23 ± 0.08	0.18 ± 0.08	<0.001

IOP = Intraocular pressure.
Both IOP and outflow facility decreased significantly after 1 week of treatment with timolol in healthy human eyes.

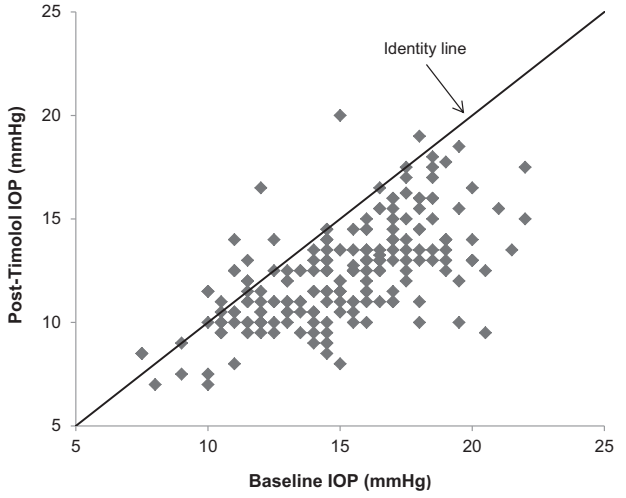

FIGURE 1. Comparison between post-timolol IOP and baseline IOP. IOP decreased from 15.1 ± 3.0 mm Hg at baseline to 12.4 ± 2.4 mm Hg after 1 week of timolol treatment ($P < 0.001$). Points below the identity line had a reduction of IOP after timolol treatment. IOP = Intraocular pressure.

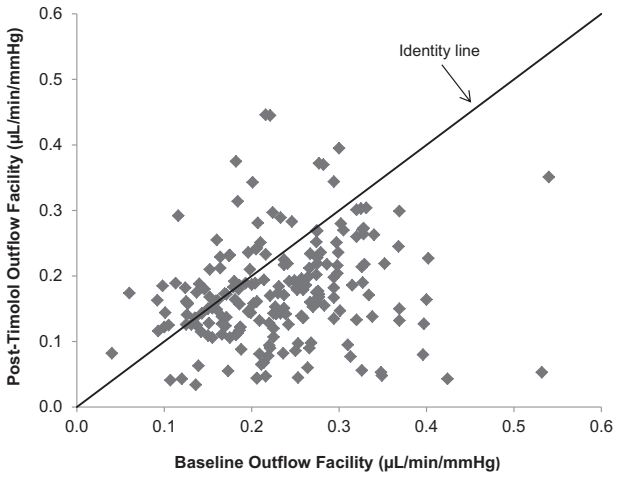

FIGURE 2. Comparison between post-timolol outflow facility and baseline outflow facility. Outflow facility decreased from 0.23 ± 0.08 μL/min/mm Hg at baseline to 0.18 ± 0.08 μL/min/mm Hg after 1 week of timolol treatment ($P < 0.001$). Points below the identity line had a reduction of outflow facility after timolol treatment.

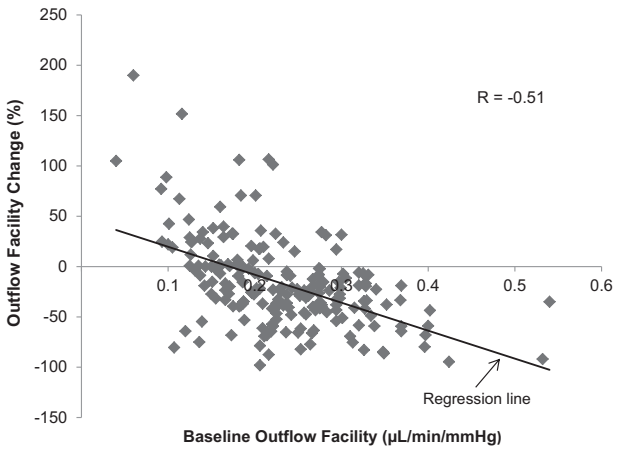

FIGURE 3. Correlation of outflow facility change with baseline outflow facility. Higher baseline outflow facility was associated with greater decrease in outflow facility after timolol treatment ($P < 0.001$).

from 0.24 ± 0.08 to 0.17 ± 0.09 μL/min/mm Hg at the University of Nebraska ($P < 0.001$).

There was a moderate strength negative correlation between baseline outflow facility and the change in outflow facility after timolol treatment ($r = -0.51$; $P < 0.001$), in which higher baseline outflow facility was associated with a greater decrease in outflow facility (Figure 3). Outflow facility decreased in 145 eyes (73%), from a mean baseline outflow facility of 0.26 ± 0.07 μL/min/mm Hg to 0.16 ± 0.07 μL/min/mm Hg after timolol treatment. In 53 eyes (26%), outflow facility increased from a mean at baseline of 0.17 ± 0.06 μL/min/mm Hg to 0.22 ± 0.09 μL/min/mm Hg after timolol treatment. Two eyes (1%) did not show any change in outflow facility.

Baseline outflow facility was weakly correlated with age in our study population ($r = -0.25$; $P < 0.001$). Older subjects were associated with lower outflow facilities (Figure 4). Baseline outflow facility was not correlated with either central corneal thickness ($r = 0.02$; $P = 0.78$) or axial length ($r = 0.07$; $P = 0.28$). Also, there were no significant differences in baseline IOP and outflow facility between men and women ($P = 0.29$ for IOP and $P = 0.07$ for outflow facility).

DISCUSSION

OUR STUDY FOUND A REDUCTION IN OUTFLOW FACILITY AFter 1 week of treatment with timolol eye drops in healthy

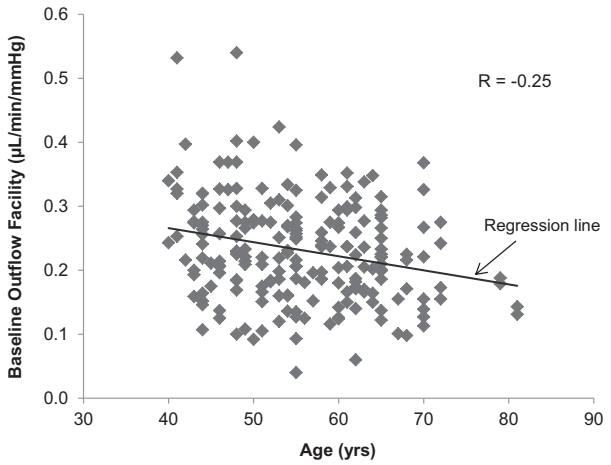

FIGURE 4. Correlation of baseline outflow facility with age. Older subjects were associated with lower outflow facilities ($P < 0.001$).

subjects. We are unaware of previous reports of this finding and could find no reference to it in a search of PubMed. Suppression of aqueous humor production is the main mechanism of action for IOP reduction by beta-adrenergic blockers such as timolol, but a decrease in outflow facility potentially limits this effect. However, the effect of timolol on aqueous humor outflow facility is variable in published studies. Although 1 animal study in cynomolgus monkeys suggested that timolol may lead to a reduction in outflow facility,[22] studies in living humans have reported no change or even an increase in outflow facility in patients with ocular hypertension or open angle glaucoma.[11–13,19,20,24–32] An important distinction, however, is that none of these studies were performed in healthy human eyes.

One possible mechanism for alteration of outflow facility by timolol in our study population might have been the blockade of beta-receptors in the trabecular outflow pathway. Thomas and Epstein[31] reported a significant increase in outflow facility after 2 weeks of treatment with epinephrine, a beta-adrenergic agonist, in 16 glaucoma patients. However, after subjects completed 2 weeks of combined therapy with timolol and epinephrine, a significant decrease in outflow facility was observed. The authors postulated that beta-adrenergic receptors mediating outflow may be located in the trabecular meshwork and that stimulating them produces an increase in outflow facility, and this effect is blocked by timolol. However, they also observed that blocking the beta-receptors with timolol alone did not decrease outflow facility in glaucoma patients. Similarly, Robinson and Kaufman[35] reported that pretreatment with timolol prevented the facility-increasing effect of topical epinephrine and norepinephrine in normotensive cynomolgus monkey eyes. They concluded that the facility-increasing effect of both epinephrine and norepinephrine was mediated by beta-2-adrenergic receptors in the trabecular meshwork endothelium. However, a single treatment with timolol alone had no effect on outflow facility in cynomolgus monkeys. This suggests that the decrease in outflow facility after 1 week of treatment in our study population may not be from an immediate effect of timolol on beta-adrenergic receptors but a slower onset mechanism of action related to aqueous humor flow suppression.

Hyposecretion of aqueous humor has been suggested to be potentially damaging to the trabecular meshwork, leading to a reduction of outflow facility.[23] Becker and Constant, in an unpublished observation of the effect of oral acetazolamide (M. Constant and B. Becker, unpublished observation, 1995), found that healthy human subjects had an initial rapid decrease in IOP but demonstrated a return toward baseline IOP after several weeks of treatment, and this was associated with a reduction in tonographic outflow facility.[23] Kiland and associates,[22] in a study of healthy cynomolgus monkeys, reported a trend toward a decrease in outflow facility after 4 weeks of treatment with timolol and dorzolamide, but this did not reach statistical significance. However, after 4 weeks of treatment with timolol plus dorzolamide plus prostaglandin, outflow facility was significantly reduced compared to that at baseline. The authors suggested that long-term use of medications that suppress aqueous humor formation, such as timolol, may cause underperfusion of the trabecular meshwork, reducing the concentration of oxygen and nutrients reaching the trabecular meshwork, possibly leading to changes that decrease outflow facility, and this effect may be exacerbated by medications that redirect aqueous humor outflow away from the trabecular meshwork, such as prostaglandin analogues. Supporting these findings, Lutjen-Drecoll and Kaufman[36] demonstrated by using electron microscopy marked histological changes in the trabecular meshwork of healthy cynomolgus monkeys after 3-7 months of topical timolol given twice daily. Furthermore, using a human anterior segment organ culture system, Johnson[37] demonstrated that perfusion rates of less than 1 µL/min over 7-21 days resulted in marked trabecular cell loss. This finding suggests that pathological changes in the trabecular meshwork as a result of severe underperfusion may result in reduction of outflow facility. However, timolol eye drops alone may not reduce aqueous humor flow rate enough to induce pathologic changes. Notably, the flow rate threshold of 1 µL/min identified by Johnson[37] is lower than the nocturnal flow rate in humans, when aqueous humor production decreases by approximately 50%[38] and lower than the flow rate typically achieved with timolol eye drops.[4,5,16–21]

Another possible explanation for the reduction in outflow facility by timolol is that changes in outflow facility in response to decreased aqueous humor flow are not pathologic but are instead pressure regulatory mechanisms. Acott and associates[39] hypothesized and reviewed the evidence that resistance within the conventional outflow pathway is continually adjusted in response to cell stretch from

sustained IOP changes. The proposed mechanism involves extracellular matrix turnover, requiring hours to days, which would be consistent with the findings in our study. This mechanism also may explain why a reduction in outflow facility was detected in our study of healthy subjects but not previous studies of glaucoma patients with presumably dysfunctional trabecular meshwork that may not be able to respond to pressure changes. Further potential evidence for a compensatory change in outflow facility in response to aqueous humor flow reduction comes from studies of circadian aqueous humor dynamics.[40–42] In healthy subjects, the reduction of aqueous humor production at night is partially compensated by a reduction in outflow facility, resulting in a relatively stable circadian IOP when measured in a constant body position. However, it is not known if the change in outflow facility is a response to the reduction in flow rate, or a concurrent change that occurs due to another mechanism.

A reduction in outflow facility with timolol treatment also may help explain the frequently observed phenomenon of tachyphylaxis. Previous studies have reported that some patients using timolol exhibited a reduction in efficacy over a few days of therapy. This clinically observed phenomenon has been termed "short-term escape."[6,7,43,44] One possible explanation for this phenomenon is the finding in a study in albino New Zealand rabbits that the number of beta-adrenergic receptors in ocular tissues increases within days of beginning timolol administration.[45] Neufeld[45] demonstrated that the number of beta receptors in ocular tissues increases with continued timolol therapy. The time course of this alteration of receptor numbers is a matter of days and would seem to correlate with the short-term escape.[45] Additionally, Johnson and associates[30] found that the degree of aqueous humor suppression decreased after 6 weeks of timolol treatment compared to 1 week of treatment. However, an increase in the number of beta receptors does not explain the decrease in outflow facility with timolol. Instead, these may be concurrent effects that both reduce efficacy in some patients.

Our study found that eyes with higher baseline outflow facility had a larger decrease in outflow facility after timolol treatment. This is consistent with the notion that healthy trabecular meshwork cells are required to produce a decreased outflow facility as a compensatory response to decreased aqueous flow rate or IOP, and this phenomenon is not a pathologic effect of hypoperfusion. Although none of our subjects had glaucoma, it is certainly possible that the spectrum of baseline outflow facilities represents a spectrum of trabecular meshwork health. Interestingly, 26% of eyes demonstrated an increase in outflow facility after treatment. These were generally eyes with lower baseline outflow facility, potentially indicating subclinical trabecular meshwork dysfunction. This is consistent with studies that demonstrated an increase in outflow facility in patients with glaucoma and ocular hypertension treated with timolol.[20,31,32] However, it is unknown what changes at the cellular level or extracellular matrix may occur that decrease flow resistance in eyes with low baseline outflow facility. It is also possible that this relationship is the result of regression to the mean, in which a group of eyes may have had outflow facility measurements lower than their normal and the increase in outflow facility after treatment in this group was a return to baseline. Further research is required to clarify the association between baseline outflow facility and outflow facility change, and the inclusion of a placebo control arm would be helpful.

Our study also found that increasing age was associated with lower baseline outflow facility in our subjects. This is consistent with the findings of most previous studies that have reported age-related decreases in tonographic outflow facility in human eyes.[46–50] However, this has not been a universal finding, as several studies reporting tonographic outflow facility in humans did not find a significant relationship between age and outflow facility.[51–53] Two other studies reported trends suggesting lower outflow facility in older subjects but did not reach statistical significance.[54,55] These apparently contradictory results may be a reflection of the inherent variability in tonography. In contrast, total outflow facility measured by 2-level constant pressure perfusion of the anterior chamber in rhesus monkeys consistently demonstrates a decrease in outflow facility with age.[56–60] It is worth noting that, although our study found a statistically significant relationship between age and baseline outflow facility, the strength of the relationship was weak in our subjects.

Compared with previous studies investigating the effect of timolol on outflow facility, our study has several important advantages. Our study had a multicenter design with a standardized protocol. This design provided a much larger sample size than all previous studies and thus was appropriately powered to detect this effect. One potential limitation of the study was that the measurements were performed by different people in different centers, and this may have resulted in interobserver variabilities. However, a subgroup analysis indicated that timolol treatment reduced outflow facility in the study populations at each site, suggesting that the measurements were consistent at each site and the results are generalizable to different healthy populations.

In summary, our study found that timolol therapy reduced aqueous humor outflow facility in eyes with healthy trabecular meshwork (as indicated by normal baseline outflow facility) after 1 week of treatment. Eyes with higher baseline outflow facility showed a greater decrease in outflow facility. Compensatory physiologic changes in the trabecular meshwork in response to reduced aqueous flow rate and intraocular pressure may provide an explanation for the decreased outflow facility in healthy eyes. However, the precise mechanism remains to be determined. The reduction in outflow facility may partially negate the overall IOP-lowering effect of timolol.

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REFERENCES

1. Quigley HA, Vitale S. Models of open-angle glaucoma prevalence and incidence in the United States. *Invest Ophthalmol Vis Sci* 1997;38:83–91.
2. Scriabine A, Torchiana ML, Stavorski JM, Ludden CT, Minsker DH, Stone CA. Some cardiovascular effects of timolol a new beta-adrenergic blocking agent. *Arch Int Pharmacodyn Ther* 1973;205:76–93.
3. Katz IM, Hubbard WA, Getson AJ, Gould AL. Intraocular pressure decrease in normal volunteers following timolol ophthalmic solution. *Invest Ophthalmol* 1976;15:489–492.
4. Dailey RA, Brubaker RF, Bourne WM. The effects of timolol maleate and acetazolamide on the rate of aqueous formation in normal human subjects. *Am J Ophthalmol* 1982;93:232–237.
5. Wayman L, Larsson LI, Maus T, Alm A, Brubaker R. Comparison of dorzolamide and timolol as suppressors of aqueous humor flow in humans. *Arch Ophthalmol* 1997;115:1368–1371.
6. Boger WP III, Puliafito CA, Steinert RF, Langston DP. Long-term experience with timolol ophthalmic solution in patients with open-angle glaucoma. *Ophthalmology* 1978;85:259–267.
7. Boger WP III, Steinert RF, Puliafito CA, Pavan-Langston D. Clinical trial comparing timolol ophthalmic solution to pilocarpine in open-angle glaucoma. *Am J Ophthalmol* 1978;86:8–18.
8. Zimmerman TJ. Timolol maleate: a new glaucoma medication? *Invest Ophthalmol Vis Sci* 1977;16:687–688.
9. Zimmerman TJ, Kaufman HE. Timolol, dose response and duration of action. *Arch Ophthalmol* 1977;95:605–607.
10. Zimmerman TJ, Kaufman HE. Timolol. A beta-adrenergic blocking agent for the treatment of glaucoma. *Arch Ophthalmol* 1977;95:601–604.
11. Zimmerman TJ, Harbin R, Pett M, Kaufman HE. Timolol and facility of outflow. *Invest Ophthalmol Vis Sci* 1977;16:623–624.
12. Zimmerman TJ, Kass MA, Yablonski ME, Becker B. Timolol maleate: efficacy and safety. *Arch Ophthalmol* 1979;97:656–658.
13. Cyrlin MN, Thomas JV, Epstein DL. Additive effect of epinephrine to timolol therapy in primary open angle glaucoma. *Arch Ophthalmol* 1982;100:414–418.
14. Camras CB. Comparison of latanoprost and timolol in patients with ocular hypertension and glaucoma: a six-month masked, multicenter trial in the United States. The United States Latanoprost Study Group. *Ophthalmology* 1996;103:138–147.
15. Watson P, Stjernschantz J. A six-month, randomized, double-masked study comparing latanoprost with timolol in open-angle glaucoma and ocular hypertension. The Latanoprost Study Group. *Ophthalmology* 1996;103:126–137.
16. Yablonski ME, Zimmerman TJ, Waltman SR, Becker B. A fluorophotometric study of the effect of topical timolol on aqueous humor dynamics. *Exp Eye Res* 1978;27:135–142.
17. Brubaker RF, Nagataki S, Bourne WM. Effect of chronically administered timolol on aqueous humor flow in patients with glaucoma. *Ophthalmology* 1982;89:280–283.
18. Coakes RL, Brubaker RF. The mechanism of timolol in lowering intraocular pressure. In the normal eye. *Arch Ophthalmol* 1978;96:2045–2048.
19. Schenker HI, Yablonski ME, Podos SM, Linder L. Fluorophotometric study of epinephrine and timolol in human subjects. *Arch Ophthalmol* 1981;99:1212–1216.
20. Lin LL, Galin MA, Obstbaum SA, Katz I. Longterm timolol therapy. *Surv Ophthalmol* 1979;23:377–380.
21. Larsson LI. Aqueous humor flow in normal human eyes treated with brimonidine and timolol, alone and in combination. *Arch Ophthalmol* 2001;119:492–495.
22. Kiland JA, Gabelt BT, Kaufman PL. Studies on the mechanism of action of timolol and on the effects of suppression and redirection of aqueous flow on outflow facility. *Exp Eye Res* 2004;78:639–651.
23. Becker B. Does hyposecretion of aqueous humor damage the trabecular meshwork? *J Glaucoma* 1995;4:303–305.
24. Kass MA, Korey M, Gordon M, Becker B. Timolol and acetazolamide. A study of concurrent administration. *Arch Ophthalmol* 1982;100:941–942.
25. Moss AP, Ritch R, Hargett NA, Kohn AN, Smith H Jr, Podos SM. A comparison of the effects of timolol and epinephrine on intraocular pressure. *Am J Ophthalmol* 1978;86:489–495.
26. Sonntag JR, Brindley GO, Shields MB. Effect of timolol therapy on outflow facility. *Invest Ophthalmol Vis Sci* 1978;17:293–296.
27. Berson FG, Epstein DL. Separate and combined effects of timolol maleate and acetazolamide in open-angle glaucoma. *Am J Ophthalmol* 1981;92:788–791.
28. Sonntag JR, Brindley GO, Shields MB, Arafat NI, Phelps CD. Timolol and epinephrine. Comparisin of efficacy and side effects. *Arch Ophthalmol* 1979;97:273–277.

29. Alexander DW, Berson FG, Epstein DL. A clinical trial of timolol and epinephrine in the treatment of primary open-angle glaucoma. *Ophthalmology* 1988;95:247–251.
30. Johnson TV, Fan S, Zhan G, Camras CB, Toris CB. Efficacy and mechanisms of intraocular pressure reduction with latanoprost and timolol in participants with ocular hypertension: a comparison of 1 and 6 weeks of treatment. *J Glaucoma* 2010; 19:356–364.
31. Thomas JV, Epstein DL. Timolol and epinephrine in primary open angle glaucoma. Transient additive effect. *Arch Ophthalmol* 1981;99:91–95.
32. Airaksinen PJ. The long-term hypotensive effect of timolol maleate compared with the effect of pilocarpine in simple and capsular glaucoma. *Acta Ophthalmol (Copenh)* 1979;57: 425–434.
33. Langham ME, Leydhecker W, Krieglstein G, Waller W. Pneumatonographic studies on normal and glaucomatus eyes. *Adv Ophthalmol* 1976;32:108–133.
34. Kazemi A, McLaren JW, Lin SC, et al. Comparison of aqueous outflow facility measurement by pneumatonography and digital schiotz tonography. *Invest Ophthalmol Vis Sci* 2017; 58:204–210.
35. Robinson JC, Kaufman PL. Effects and interactions of epinephrine, norepinephrine, timolol, and betaxolol on outflow facility in the cynomolgus monkey. *Am J Ophthalmol* 1990;109:189–194.
36. Lutjen-Drecoll E, Kaufman PL. Long-term timolol and epinephrine in monkeys. II. Morphological alterations in trabecular meshwork and ciliary muscle. *Trans Ophthalmol Soc U K* 1986;105(Pt 2):196–207.
37. Johnson DH. Human trabecular meshwork cell survival is dependent on perfusion rate. *Invest Ophthalmol Vis Sci* 1996; 37:1204–1208.
38. Brubaker RF. Flow of aqueous humor in humans. The Friedenwald Lecture. *Invest Ophthalmol Vis Sci* 1991;32: 3145–3166.
39. Acott TS, Kelley MJ, Keller KE, et al. Intraocular pressure homeostasis: maintaining balance in a high-pressure environment. *J Ocul Pharmacol Ther* 2014;30(2–3):94–101.
40. Liu H, Fan S, Gulati V, et al. Aqueous humor dynamics during the day and night in healthy mature volunteers. *Arch Ophthalmol* 2011;129:269–275.
41. Nau CB, Malihi M, McLaren JW, Hodge DO, Sit AJ. Circadian variation of aqueous humor dynamics in older healthy adults. *Invest Ophthalmol Vis Sci* 2013;54:7623–7629.
42. Sit AJ, Nau CB, McLaren JW, Johnson DH, Hodge D. Circadian variation of aqueous dynamics in young healthy adults. *Invest Ophthalmol Vis Sci* 2008;49:1473–1479.
43. Boger WP 3rd. Timolol: short term "escape" and long term "drift". *Ann Ophthalmol* 1979;11:1239–1242.
44. Boger WP 3rd, Steinert RF, Thomas JV. Timolol in the therapy of "ocular hypertension". *Surv Ophthalmol* 1980;25: 195–202.
45. Neufeld AH, Zawistowski KA, Page ED, Bromberg BB. Influences on the density of beta-adrenergic receptors in the cornea and iris-ciliary body of the rabbit. *Invest Ophthalmol Vis Sci* 1978;17:1069–1075.
46. Becker B. The decline in aqueous secretion and outflow facility with age. *Am J Ophthalmol* 1958;46(5 Part 1):731–736.
47. Gaasterland D, Kupfer C, Ross K, Gabelnick HL. Studies of aqueous humor dynamics in man. 3. Measurements in young normal subjects using norepinephrine and isoproterenol. *Invest Ophthalmol* 1973;12:267–279.
48. Gaasterland D, Kupfer C, Milton R, Ross K, McCain L, MacLellan H. Studies of aqueous humour dynamics in man. VI. Effect of age upon parameters of intraocular pressure in normal human eyes. *Exp Eye Res* 1978;26:651–656.
49. Croft MA, Oyen MJ, Gange SJ, Fisher MR, Kaufman PL. Aging effects on accommodation and outflow facility responses to pilocarpine in humans. *Arch Ophthalmol* 1996;114:586–592.
50. Weekers R, Watillon M, De Rudder M. Experimental and clinical investigations into the resistance to outflow of aqueous humour in normal subjects. *Br J Ophthalmol* 1956; 40:225–233.
51. Grant WM. Clinical measurements of aqueous outflow. AMA *Arch Ophthalmol* 1951;46:113–131.
52. De Roetth A Jr, Knighton WS. Clinical evaluation of the aqueous-flow test; a preliminary report. AMA *Arch Ophthalmol* 1952;48:148–153.
53. Boles-Carenini B, Cambiaggi A. Are aqueous humor dynamics influenced by aging. *Am J Ophthalmol* 1957;44: 395–401.
54. Spencer RW, Helmick ED, Scheie HG. Tonography; technical difficulties and control studies. AMA *Arch Ophthalmol* 1955;54:515–527.
55. Toris CB, Yablonski ME, Wang YL, Camras CB. Aqueous humor dynamics in the aging human eye. *Am J Ophthalmol* 1999; 127:407–412.
56. Gabelt BT, Crawford K, Kaufman PL. Outflow facility and its response to pilocarpine decline in aging rhesus monkeys. AMA *Arch Ophthalmol* 1991;109:879–882.
57. Gabelt BT, Kaufman PL. Changes in aqueous humor dynamics with age and glaucoma. *Prog Retin Eye Res* 2005;24: 612–637.
58. Kiland JA, Croft MA, Gabelt BT, Kaufman P. Atropine reduces but does not eliminate the age-related decline in perfusion outflow facility in monkeys. *Exp Eye Res* 1997;64: 831–835.
59. Kiland JA, Gabelt BT, Kaufman PL. Effect of age on outflow resistance washout during anterior chamber perfusion in rhesus and cynomolgus monkeys. *Exp Eye Res* 2005;81: 724–730.
60. Kiland JA, Gabelt BT, Kaufman PL. Relationship of aqueous outflow resistance to age and total volume perfused in rhesus and cynomolgus monkeys. *Invest Ophthalmol Vis Sci* 2011;52: 6820–6824.