Calibration of the TonoLab Tonometer in Mice with Spontaneous or Experimental Glaucoma

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Purpose. To measure the accuracy of TonoLab (TioLat, Helsinki, Finland) tonometry in mice with spontaneous or induced experimental glaucoma.

Methods. Chronic intraocular pressure (IOP) elevation was induced in one eye of 32 mice by injection of polystyrene beads and viscoelastic material. Three to 6 weeks later, the eyes were cannulated and manometrically set to 10, 20, 30, 40, or 50 mm Hg. The mice were 8-week and 8-month-old C57BL/6, 8-week-old DBA/2J, and 8-week-old CD1. The TonoLab calibration was also tested on five aged DBA/2J mice with spontaneous glaucoma. The relation of the TonoLab reading to manometric IOP was evaluated in multivariate linear regression models with axial length, IOP history, and mouse strain as independent variables.

Results. The slope of the relationship between TonoLab and manometric IOP in all the mice was 0.998, with an intercept of 2.3 mm Hg (adjusted R in univariate regression = 0.86). Neither the mice with bead-induced glaucoma nor those with spontaneous glaucoma (older DBA/2J mice) differed significantly from the control animals in having an excellent correlation between TonoLab and manometer IOP. Longer and wider mouse eyes had slightly higher tonometrically measured IOP, whether glaucomatous or control (multivariate regression, adjusted $R^2 = 0.90$, $P < 0.0001$). There was no difference in tonometric accuracy among the three mouse strains: CD1, C57BL/6, and DBA/2J, nor between 8-week and 8-month-old C57BL/6 mice (multivariate regression, $P = 0.32$).

Conclusions. The TonoLab accurately reflects IOP in both normal mice and in eyes of mice with experimental or spontaneous glaucoma, with no detectable effect of age. (*Invest Ophthalmol Vis Sci.* 2011;52:858–864) DOI:10.1167/iovs.10-5556

It is essential to measure intraocular pressure (IOP) accurately in experimental glaucoma research. Mouse models permit genetic manipulations in the elucidation of glaucoma pathogenesis and treatment, but the tiny size of the mouse's eye presents a challenge to accurate tonometry. Various solutions have been devised for IOP measurement in the mouse, including needle cannulation through the cornea, use of existing commercial tonometers such as the pneumatonograph and TonoPen (Reichert, Inc., Depew, NY), and modifications of other commercial tonometers.[1–10]

The validity of noninvasive tonometry in the mouse eye has been confirmed in several strains under anesthesia.[11–14] McKinnon et al.[15] concluded: "TonoLab tonometry is technically easier than TonoPen tonometry, and has become the IOP measurement technique of choice" for experimental glaucoma in the mouse. In the DBA/2J mouse, spontaneous increase in IOP occurs, and many experiments have been conducted, often with needle cannulation used for tonometry. It may be preferable not to puncture the cornea repeatedly to monitor IOP, but it has not been shown that tonometry accurately reflects IOP in spontaneous or induced glaucoma in the mouse. Continuous IOP monitoring with implantable devices is under development, but has not been reported for mouse eyes.

Sappington et al.[16] have recently published a mouse model of glaucoma involving injection of beads into the anterior chamber. We have modified this model by first injecting beads (of 6 μm diameter) followed by viscoelastic injection. In a companion report, we confirm that the mouse eye enlarges significantly with chronic IOP elevation, as was first documented in the DBA/2J mouse. Enlargement of the eye could alter tonometric accuracy. Hence, we performed calibration studies in mouse eyes with bead/viscoelastic experimental glaucoma and their fellow eyes, studying younger and older mice of three different strains, as well as DBA/2J mice after development of spontaneous glaucoma. One previous study compared TonoLab (TioLat, Helsinki, Finland) IOP measures with needle cannulation, but without testing a range of IOP.[17] To our knowledge, this is the first extensive calibration study of tonometry in both spontaneous and induced mouse glaucoma.

Methods

Animals

A total of 37 female mice were used in the study. Eight mice were 8-week-old C57BL/6 (seven eyes with bead-induced glaucoma, six control eyes), eight were 8-month-old C57BL/6 (five eyes with bead-induced glaucoma and four control eyes had calibration results), eight were 8-week-old DBA/2J (seven eyes with bead-induced glaucoma and seven control eyes had calibration results), and eight were 8-week-old CD1 (seven eyes with bead-induced glaucoma and eight control eyes had calibration results). Five DBA/2J mice were also studied at age 13 to 24 months (eight eyes had calibration results) after development of spontaneous glaucoma. Four mice died of complications of the anesthesia, and one was euthanatized because of severe ocular enlargement with corneal ulceration, leaving a total of 32 mice that provided data. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, using protocols approved and monitored by the Johns Hopkins University School of Medicine Animal Care and Use Committee.

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Supported in part by Public Health Service research Grants EY02120 (HAQ) and EY01765 (Wilmer Institute Core Grant) and by the Leonard Wagner Trust and William T. Forrester.

Submitted for publication March 19, 2010; revised May 17 and June 23, 2010; accepted July 24, 2010.

Disclosure: **M.E. Pease,** None; **F.E. Cone,** None; **S. Gelman**, None; **J.L. Son,** None; **H.A. Quigley**, None

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Bead Injection for Induction of Experimental Glaucoma

The mice were anesthetized by intraperitoneal injection of ketamine, xylazine, and acepromazine (50, 10, and 2 mg/kg, respectively). One eye was proptosed, and a glass cannula with tip diameter of 50 μm was inserted into the inferior portion of the anterior chamber, with injection of 2 μL of 6-μm beads (Polybead Microspheres; Polysciences, Inc., Warrington, PA), followed by 3 μL of viscoelastic solution (10 mg/mL hyaluronate sodium; Healon; Advanced Medical Optics Inc., Santa Ana, CA) through a syringe (Hamilton, Inc., Reno, NV). The beads were sterilized before injection by placing them in 100% ethanol in 0.5-mL tubes (Eppendorf, Fremont, CA). They were then centrifuged, resuspended in alcohol, and washed twice in sterile phosphate-buffered saline with recentrifugation. The final pellet was aspirated directly into the glass micropipette used for injection (at 3×10^6 beads per microliter). The needle was left in place for 2 minutes, to minimize efflux of injected material.

During the development of induced glaucoma from bead injection, IOP measurements were made in both eyes with the TonoLab tonometer (TioLat), with mice under both topical anesthesia with 0.5% proparacaine hydrochloride eyedrops and general anesthesia (as described earlier). IOP was measured before bead injection, 3 days after injection, and weekly thereafter, until the terminal calibration experiments at 3, 4, 5, or 6 weeks after injection. The TonoLab acquires six valid rebounds of the device from the eye and takes the mean of the middle four readings to make one summary measurement. The instrument indicates the reliability of the reading by the position of a bar next to the reported value. A single set of readings was collected with the best reproducibility indicator (no bar) at each time point during the periodic IOP measurements before calibration studies.

Calibration of TonoLab

For the final calibration study, mice were anesthetized as for bead injection, and an additional 50-mg/kg dose of ketamine was given when needed to maintain immobility. Both eyes were cannulated after a pilot hole had been created in the superotemporal, peripheral cornea with a 30-gauge needle. Then, a short segment of 30-gauge needle that had been removed from its plastic hub and polished smooth was inserted into the anterior chamber, connected by a short segment of polyethylene (PE 10) tubing to a stopcock that was connected to a reservoir of balanced salt solution containing 0.1% heparin. The blunt cannula and tubing for each eye was carefully positioned over the head and lightly taped to avoid applying weight and tension to the eye. The eye was therefore freely moveable during tonometry. The height of the reservoir was adjusted to determine IOP, and the stopcock was open from the eye to the reservoir during measurement. The first readings were taken 15 minutes after cannulation, to allow for initial equilibration of IOP.

The TonoLab requires an optimal tear film to obtain a reliable reading, as the probe may stick to either a dry cornea or to excess tear fluid. This event and other oblique rebounds are not counted as measurements and generally are signaled by the instrument as an error reading. To address this limitation, we moistened the cornea with balanced salt solution (BSS; Alcon, Fort Worth, TX) and blotted between each series of readings. This process was especially necessary with the glaucomatous eyes, as their corneas tended to dry more quickly than control eyes. We collected five means of six measurements each from the eye at each IOP setting (a total of 30 measurements per eye) and calculated the overall mean from the middle three mean values. In this portion of the study, we used TonoLab readings with either the best reproducibility (no bar symbol) or the next best reproducibility (one bar at the bottom of the display window). After each data collection at a pressure level, the bottle height was moved to the next pressure level, and 10 minutes was allowed for equilibration between the anterior chamber and the reservoir. Readings were taken stepwise at reservoir heights corresponding to pressures of 10, 20, 30, 40, and 50 mm Hg. Prior mouse calibration experiments have demonstrated that the direction of IOP change (low to high compared with high to low) is significantly related to calibration accuracy.[12]

Axial Length Measurements

Animals were killed by overdose with intraperitoneal injection of ketamine, xylazine, and acepromazine (50, 10, and 2 mg/kg, respectively). Both eyes were enucleated and fixed by immersion in 4% paraformaldehyde in 0.1 M Na_3PO_4. The enucleated eyes were cleaned of extraocular tissue and manually inflated to 15 mm Hg with a needle inserted into the anterior chamber connected to a reservoir of saline that determined the IOP before measurement of axial length and width with a digital caliper (Instant Read Out Digital Caliper; Electron Microscopy Sciences, Hatfield, PA). The length was measured from the center of the cornea to a position just temporal to the optic nerve, and width was measured at the largest dimension at the equator, midway between the cornea and optic nerve.

Optic Nerve Axon Assessment

After overnight fixation in paraformaldehyde, the optic nerves were removed, and both globes and optic nerves were postfixed in 1% osmium, dehydrated in graded alcohol, and stained in 1% uranyl acetate in 100% ethanol for 1 hour before infiltration and embedding in epoxy resin. One-micrometer-thick cross sections were taken of the optic nerve and stained with 1% toluidine blue in 1% sodium borate. The degree of damage to axons in each optic nerve of bead-induced glaucoma and spontaneous DBA/2J glaucoma, as well as the number in fellow control eyes of animals with bead-induced glaucoma was judged by masked observers by quantitative assessment. Digital images of the nerves were taken at low power, to measure the total optic nerve area in each nerve and then at 100× (Cool Snap camera and image-analysis software; Roper Scientific, Tucson, AZ; Metamorph, Universal Imaging, Downingtown, PA). For each nerve, five 40 × 40-μm fields were acquired, equaling a 9% sample of the total nerve area. A masked observer edited nonaxonal elements from each image, generating an axon density measure. Average axon density/per square millimeter was multiplied by the individual nerve area to estimate the number of axons. Nerve area and density were calculated for each nerve. The mean number of axons in pooled, fellow eye nerves of the appropriate strain, length of glaucoma, and tissue fixation was compared to that in the experimental eye to yield the percentage of axon loss. For older DBA/2J nerves in which both eyes were expected to be abnormal, the control was young DBA/2J normal eyes.

Statistical Methods

Data for relevant variables were compared by standard univariate and multivariate linear regression analyses (models; SAS software; SAS, Cary, NC). In these analyses, the dependent variable was typically tonometer reading, and the independent variables included one or more of the following: manometer setting (10–50 mm Hg), age, strain, width or length of globe, presence of bead-injection glaucoma, and presence of spontaneous DBA/2J glaucoma. We adjusted for the inclusion of both eyes of some mice through random-effects modeling, and analyses included: (1) all mouse eyes, (2) only control eyes, (3) only eyes with bead-induced glaucoma, (4) only eyes with spontaneous DBA/2J glaucoma, and (5) comparisons of young and old C57BL/6 mice.

Results

By univariate linear regression, with 310 observations of IOP in 32 mice, we found an excellent correlation between IOP as measured by the TonoLab and manometer-set IOP (Table 1). There was a high level of correlation in similar analyses that included either all mouse eyes, only control eyes, only eyes with bead-induced glaucoma, and only DBA/2J older eyes with spontaneous glaucoma (Table 1). All groups of mice had a slope of nearly 1 and intercepts from 0 to 3 mm Hg, indicating

Table 1. Comparisons of TonoLab-Measured IOP to Manometrically Set Pressure by Univariate Linear Regression

Treatment Group	Slope	Intercept	Adjusted R^2
All mice	0.998	2.3	0.86
Control only	0.978	1.3	0.95
Bead-induced glaucoma only	1.013	3.4	0.78
DBA/2J glaucoma only	0.992	2.6	0.96

Table 2. Tonometer IOP versus Manometer Pressure Comparing Control Eyes with Two Groups of Glaucomatous Eyes by Multivariate Linear Regression

Independent Variables	*P*
Intercept on *y*-axis	0.28
Manometer level of IOP	<0.0001
Bead-induced glaucoma	0.20
DBA/2J glaucoma	0.60

that the tonometer tracked the set IOP just above the ideal agreement line, exceeding set IOP by ≤5%. (The intercept on the y-axis indicates the overestimation by tonometry at low IOP. and if the regression slope is close to 1, it is a fair estimate of the general overestimation across the tested range of IOP.) The correlations in all control eyes and in all eyes with bead-induced glaucoma are shown graphically in Figure 1.

Using multivariate linear regression, we examined the relationship of TonoLab-measured IOP to manometer-set IOP, as well as the potential effect that the presence of either bead-induced or spontaneous glaucoma might have on the tonometer–manometer comparison. We performed a multivariate linear regression analysis with tonometer IOP as the dependent variable and the following independent variables: manometer-set IOP, bead-induced glaucoma, and DBA/2J glaucoma. (Each glaucoma group was compared to control eyes as the reference.) Such a linear regression model can be plotted as the relationship of TonoLab-measured IOP on the *y*-axis and manometer-set IOP on the *x*-axis, where the ideal result would be that the slope would be 1 and the intercept of the line on the *y*-axis would be at 0. The analysis calculates the intercept (of the *y*-axis) as a term, and when $P > 0.05$, we conclude that the intercept is not significantly greater than 0. If the glaucomatous eyes were different from control eyes, the terms for bead-induced glaucoma or DBA/2J glaucoma would be significantly different from the reference group of control subjects. This linear regression analysis showed a highly significant relationship between the tonometric and manometric IOP ($P < 0.0001$), but no effect of either bead-induced glaucoma or spontaneous glaucoma on the tonometric IOP (Table 2). The low degree of variability between tonometric and manometric IOP is indicated by the high adjusted R^2 value of 0.86 for this analysis. The intercept of the regression line on the *y*-axis had a value of 1.3 mm Hg, indicating a slight overestimation of manometrically set IOP by the TonoLab, but this was not significantly greater than a 0 intercept ($P = 0.28$).

In another analysis, we compared only the control and bead-induced glaucoma eyes (without the DBA/2J spontaneous group) with a nearly identical result. A further analysis found no effect of duration of induced glaucoma (from 3 to 6 weeks) on the correlation between tonometric IOP and manometric IOP ($P = 0.52$, multivariate linear regression). There was no statistically significant difference in the intercept between bead-injected and control eyes, nor between eyes with spontaneous DBA/2J glaucoma and control eyes, with the average intercept at 1.29 mm Hg.

The linear regression relationship of TonoLab to manometric IOP in DBA/2J eyes with spontaneous glaucoma alone is shown in Figure 2.

Eye size significantly affected the relationship between TonoLab and manometer pressure as shown by multivariate

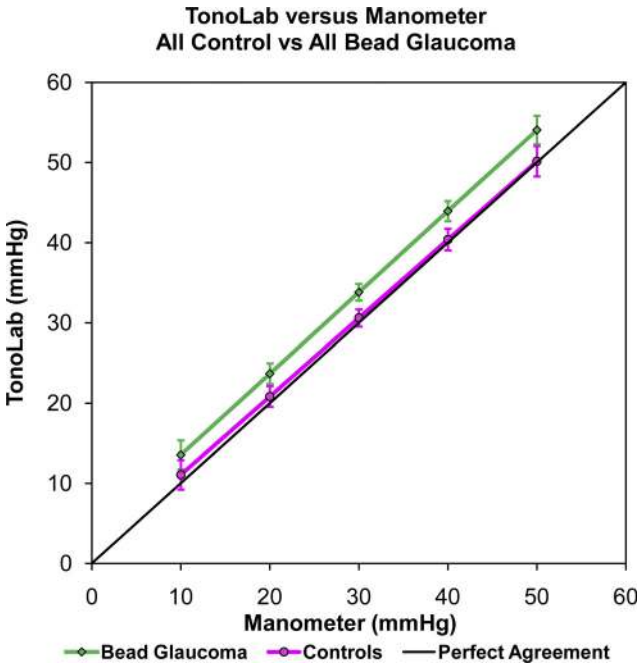

Figure 1. Linear regression of eyes with bead-induced glaucoma and control eyes shows a high level of correlation between manometric IOP and TonoLab (TioLat, Helsinki, Finland) readings at each setting. *Solid black line*: perfect agreement between the TonoLab and manometric-set IOP. Error bars, 95% CI for each data point.

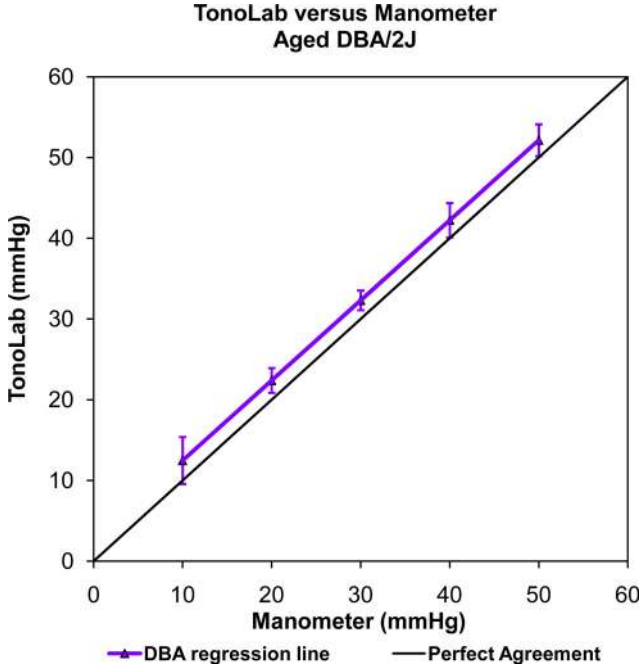

Figure 2. Linear regression of DBA/2J eyes with spontaneous glaucoma shows a high level of correlation between manometric IOP and TonoLab (TioLat, Helsinki, Finland) readings at each setting. *Solid black line*: Perfect agreement between the TonoLab and manometric-set IOP. Error bars, 95% CI for each data point.

TABLE 3. Axial Length Increase in Eyes with Bead-Induced or Spontaneous Glaucoma

Treatment Group	*n*	Control	Bead-Induced	% Increase	*P**
C57BL/6 younger	8	3.24 ± 0.25	3.74 ± 0.28	16	0.002
C57BL/6 older	8	3.55 ± 0.19	3.82 ± 0.23	8	0.02
DBA/2J younger	8	3.45 ± 0.22	3.70 ± 0.15	8	0.02
CD1 younger	8	3.46 ± 0.15	3.67 ± 0.34	6	0.13
DBA/2J older, glaucoma	5		3.89 ± 0.15	13†	0.0004

Data are expressed in mean millimeters ± SD.
* Difference between bead-induced and control.
† Older DBA/2J compared with young DBA/2J control eyes.

linear regression, although the tonometric accuracy remained significant when eye size was an independent variable. In this multivariate linear regression analysis, tonometric IOP was the dependent variable, and there were two independent variables: manometrically set IOP and axial length (both were significantly related to tonometric IOP at $P < 0.0001$). The effect of eye size on the tonometer–manometer correlation was independent of the presence of glaucoma; that is, it was seen in control eyes as well. Both longer eyes and wider eyes, as measured postmortem at 15 mm Hg, had significantly, but only slightly higher tonometric IOP at any set manometric pressure ($P < 0.0001$).

The difference in lengths between eyes with induced glaucoma and their fellow control eyes is shown in Table 3. Although these bead-injected eyes had been observed for 3 to 6 weeks, their elongation was close to that in eyes from a prior study with this model in which eyes were monitored for up to 12 weeks.[18] The length of older DBA/2J eyes with spontaneous glaucoma was significantly greater than that of control, young DBA/2J eyes (Table 3). The control, older C57BL/6 mice had significantly longer eyes than those of the control, younger mice of the same strain ($P = 0.01$, *t*-test). Younger C57BL/6 eyes showed no significant difference in control eye axial length from younger DBA/2J or younger CD1 control eyes ($P = 0.10$, $P = 0.06$, respectively).

We next evaluated whether the level of manometrically set IOP was related to the degree to which the TonoLab slightly overestimates IOP. Because we used a protocol in which IOP was set initially at 10 mm Hg and increased in 10-mm Hg increments, this variable captured not only potential influence of IOP height, but also any influence of the order of measurement during the experiment. This variable was not significantly related to the TonoLab–manometer difference in linear regression models that included length or width of the eye, age, and strain ($P = 0.42$ for control eyes, 0.96 for induced-glaucoma eyes). Since eye width and length were slightly but significantly related to the small TonoLab overestimation of set IOP, we divided all eyes in the study (control and glaucoma) into those higher and lower than the median width value of 3.37 mm. The slope for the difference between tonometer and manometer IOP (*y*-axis) versus level of manometer IOP (*x*-axis) was different in the larger eyes than in the smaller eyes ($P = 0.04$; Table 4, Fig. 3). Thus, larger eyes had higher tonometric IOPs at higher manometric IOPs than did smaller eyes, although the mean disparity from set IOP as a percentage of actual IOP, even in the most extreme category (larger eyes at highest IOP), was only 10% (<5 mm Hg too high at a set IOP of 50 mm Hg).

We found that the three mouse strains (DBA/2J, C57BL/6, and CD1) did not differ significantly in the relationship between TonoLab IOP and manometer IOP. This finding was true whether considering only control nonglaucomatous eyes, or eyes with induced or spontaneous glaucoma. For example, using multivariate linear regression with tonometer IOP as the dependent variable and taking into account mouse strain, the tonometer–manometer relationship remained highly significant ($P < 0.0001$; Table 5). This analysis (Table 5) included both bead glaucoma eyes and normal fellow eyes (but not DBA/2J older mice with glaucoma). This multivariate linear regression analysis included as independent variables: axial length, slope of tonometer–manometer relation by strain, and intercept on the *y*-axis by strain. Neither slope differ-

TABLE 4. Tonometer–Manometer Difference Compared with Manometer IOP Level by Multivariate Linear Regression

Independent Variables	*P*
Intercept	0.03
Manometer level of IOP	0.14
Intercept difference: larger vs. smaller	0.56
Slope difference: larger vs. smaller	0.04

Larger, width ≥3.37 mm; smaller, width <3.37 mm.

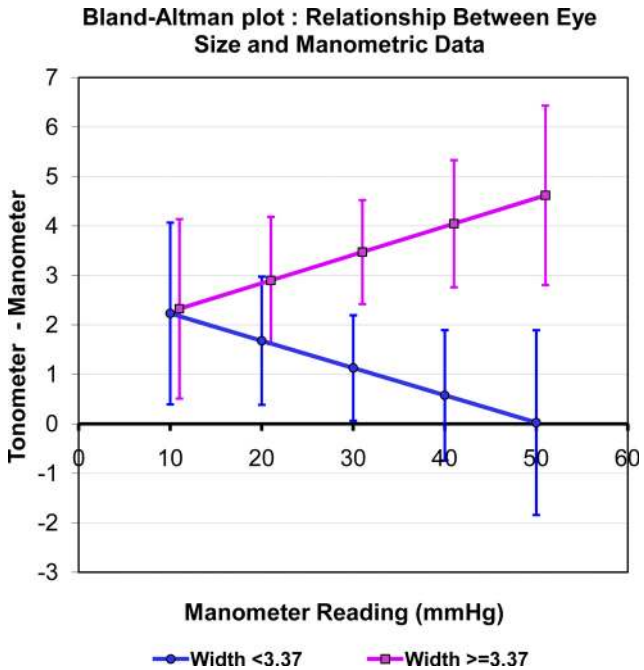

FIGURE 3. A Bland-Altman plot to examine whether the difference between tonometric and manometric IOP (*y*-axis) is related to the manometric IOP level (*x*-axis), with a comparison of eyes divided into two groups by the median eye width of 3.37 mm. Eyes smaller than 3.37 mm (*blue line*, *circles*) had an overestimation of manometric IOP by tonometry that decreased with increasing IOP, whereas the larger eyes (>3.37 mm) had a modest increase in the tonometric overestimation at higher set IOP ($P = 0.04$). Even at the greatest disparity, the larger eye group was within 10% of set IOP.

TABLE 5. Influence of Mouse Strain on Tonometric–Manometric Correlation in Multivariate Linear Regression Analysis

Independent Variables	*P*
Manometer level of IOP	<0.0001
Axial length	<0.0001
Intercept difference, CD1 vs. C57BL/6	0.58
Intercept difference, DBA/2J vs. C57BL/6	0.27
Slope difference, CD1 vs. C57BL/6	0.51
Slope difference, DBA/2J vs. C57BL/6	0.34

ence nor intercept difference was statistically significant between strains.

When we compared age as an independent variable that might affect tonometric accuracy by multivariate linear regression, 2- and 8-month-old C57BL/6 mice (both bead and control) did not differ significantly (adjusted $R^2 = 0.79$, difference by age variable $P = 0.32$; Fig. 4).

The mean IOP over 5 weeks in bead-injected and fellow control eyes included in this analysis is shown in Figure 5. The differences between experimental glaucoma eyes and their fellow control eyes were statistically significant at all time points (all $P < 0.004$ or greater, *t*-test).

Of the 28 mice that had bead-induced glaucoma in one eye, 25 had optic nerve cross sections that were gradable for degree of axon loss (three nerves had inadequate preservation for quantification). The axon counts were: C57BL/6 young: range, no loss to 40%; mean loss (± SD), 14.6% ± 15.9% versus matched controls, $n = 7$; C57BL/6 older: range, no loss to 18%; mean loss, 1.0% ± 11.6%; $n = 5$; DBA/2J young: range, no loss to 14%; mean loss, 3.8% ± 8.1%; $n = 7$; CD1 young: range, no loss to 64%; mean loss, 2.1% ± 33.2%; $n = 6$; and DBA/2J older: range, no significant loss to 95% loss, mean loss, 42.2% ± 47.0%, $n = 7$ eyes of five animals (Fig. 6). There was no significant relationship between degree of axon loss and the tonometric–manometric relation for glaucomatous eyes ($P > 0.05$).

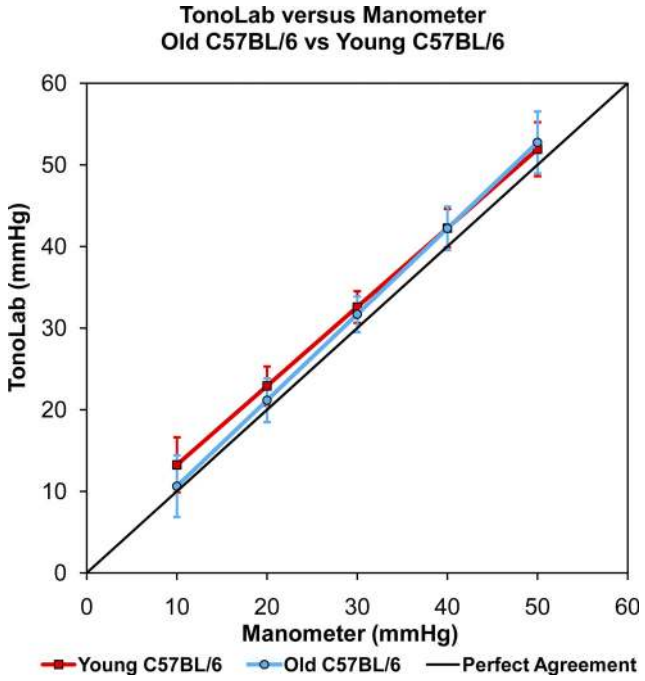

FIGURE 4. Linear regression of tonometer IOP compared with manometer-set IOP in older compared with younger C57BL/6 mice (combined control and bead-induced glaucoma eyes) shows that age does not have a statistically significant effect on the accuracy of the TonoLab (TioLat, Helsinki, Finland). *Solid black line*: Perfect agreement between the TonoLab and manometric-set IOP. Error bars, 95% CI for each data point.

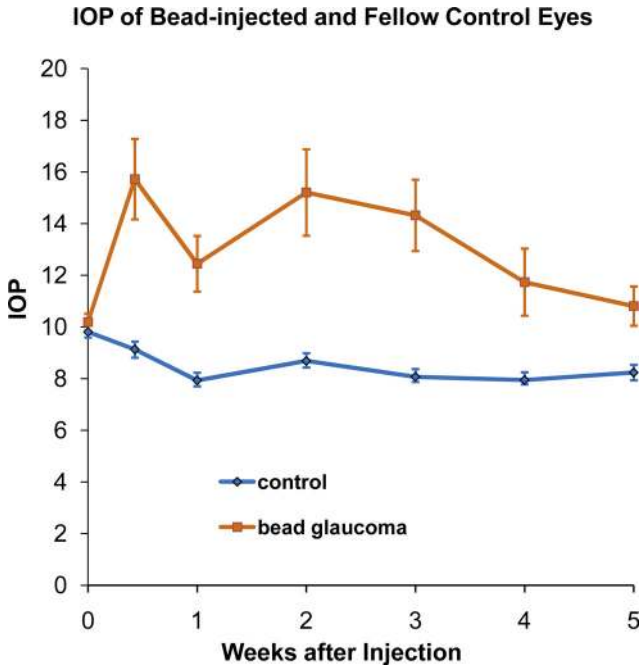

FIGURE 5. IOP of bead-injected and fellow control eyes. Experimental glaucomatous eyes had significantly higher IOP at each measured time point after baseline ($P < 0.004$ or greater, *t*-test). Animals measured under anesthesia with TonoLab. Number of mice at each time point in each group was: 29 eyes for 3 days, 1 and 2 weeks, 28 eyes at 3 weeks, 18 at 4 weeks, and 21 at 5 weeks. Data are the mean ± SEM.

DISCUSSION

When rodent glaucoma models were first developed more than a decade ago, various methods were attempted for measuring IOP. The tonometers designed for humans were used in rats, with multiple measurements by the TonoPen (Reichert, Inc.) becoming a widely used method. In a direct comparison, we found that normal and glaucomatous rat eyes were more accurately measured with the TonoLab (TioLat) than the TonoPen.[12] Since the mouse eye is nearly 10 times smaller than the human eye, it is even less likely that the TonoPen (or other tonometers designed for human eyes) would provide accurate measurements. Investigators who wanted to study the DBA/2J mouse model of spontaneous glaucoma developed needle-puncture methods to estimate IOP. Avila et al.[2] developed a servo-null method that also involved needle puncture of the cornea with transducer measurement of IOP. Although clearly accurate, this method is less desirable for repeated measurements and, as described by Avila et al.,[2] is expensive and requires specialized training for performing the measurements. Furthermore, with induced glaucoma models, such as outflow obstruction with microbead injection,[16] repeated corneal puncture could allow exit of beads and decrease model efficiency. We previously found that the TonoLab accurately reflected normal mouse eye pressure in vivo in cannulation studies and was superior to the TonoPen in rat glaucoma eyes, but it had not been shown that the TonoLab would be accurate in experimental glaucoma mice or DBA/2J mice with spontaneous glaucoma.[19]

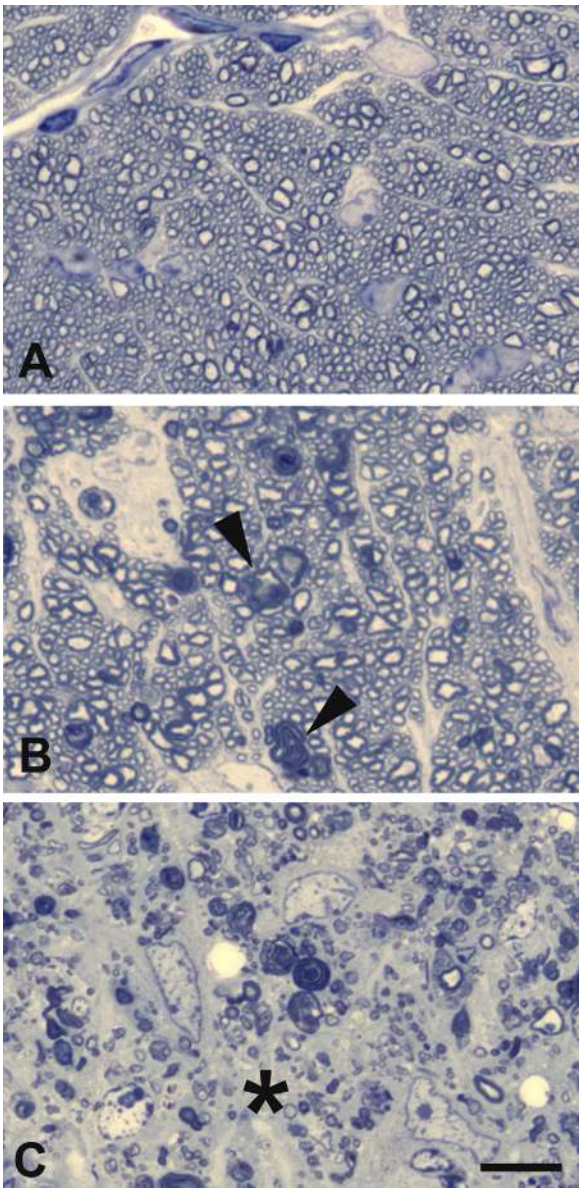

FIGURE 6. Representative images of optic nerve cross sections. (**A**) A section from a bead-injected, young DBA mouse with an axon count of 49,758, showing no damage. (**B**) A section showing moderate damage from an older C57/Bl6 mouse with 36,367 fibers remaining. An optic nerve from an aged DBA/2J mouse with spontaneous glaucoma and only 7880 fibers remaining is shown in (**C**). (**B**, *arrowheads*) degenerating axons; (**C**, ✱) areas of severe atrophy. Bar, 10 μm.

Our present results show that good estimates of IOP are attained not only in mice with bead-induced glaucoma, but in DBA/2J mice that have developed glaucomatous injury. We are presently studying strain differences in susceptibility to glaucomatous damage among the three strains of mice studied and have been pleased to see that IOP in each of these strains is accurately measured. Filippopoulos et al.[17] reported a calibration curve for normal, enucleated mouse eyes for TonoLab compared with manometrically set IOP. We and others have found it practical and clearly preferable to calibrate tonometers in the living animal, to evaluate instruments in eyes with normal position in the orbit and with normal blood flow. Filippopoulos et al. also measured a group of DBA/2J mouse eyes with TonoLab and then sequentially used the needle-puncture transducer method to measure the IOP. No TonoLab measurements were made with manometrically set IOP, and the range of IOPs compared was only below 20 mm Hg. Although their methods were therefore different from our own, they concluded that the TonoLab was useful in IOP measurements in DBA/2J mice. Wang et al.[20] conducted cannulation calibration of the TonoLab in three normal BALB/c mouse eyes at seven levels of manometrically set IOP. There was an excellent correlation; however, no glaucomatous mouse eyes were studied. To our knowledge, the present report is the first in which such a calibration was performed on glaucomatous mouse eyes.

The finding that TonoLab slightly overestimates manometric IOP is consistent with human studies in which the Icare (Icare Finland, Ltd., Helsinki, Finland) tonometer (the human version of TonoLab) was compared with Goldmann applanation tonometry. In such studies, Icare measurements were highly correlated as well as slightly higher than Goldmann applanation tonometry readings.[21,22]

Scholz et al.[23] found no strong correlation between the pattern of IOP as measured by TonoLab over time and degree of optic nerve damage in DBA/2J animals; however, they did not provide data on mean IOP over time and did not quantify optic nerve axons or RGC body damage. Inman et al.[24] found an excellent correlation between axon counts provided in detail and TonoPen measures in DBA/2J animals. We studied quantitative optic nerve axon loss and retinal ganglion cell loss in a large number of mice with bead-induced glaucoma in one eye (Cone F, personal communication, 2010). The IOP exposure over time was measured weekly for 6 to 12 weeks but correlated weakly with loss of RGCs and their axons. With the data provided herein, we can now rule out tonometric inaccuracy as the cause of this low correlation. It may be that inadequate sampling of IOP during chronic mouse glaucoma experiments is a more likely explanation.

It would be ideal to have a method of measuring IOP without anesthesia in rats and mice, since anesthesia lowers IOP in both species.[25] Successful training of rats and mice to accommodate to tonometry is time consuming and strain dependent. A description of a restraining device for measurement of IOP in awake mice was published by Nissirios et al.[26] We and others have found that some mice fight this apparatus vigorously, raising IOP artificially[27] in the process.

In summary, the TonoLab tonometer was calibrated in living mice with two forms of glaucoma, demonstrating that it accurately reflects the true IOP, as has been demonstrated in normal mouse eyes.

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