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Manipulation of intraocular pressure for studying the effects on accommodation☆

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ABSTRACT

A reliable experimental system in which IOP can be manipulated or a rapid IOP change can be induced while simultaneously and continuously measuring IOP and the ocular accommodative changes would be useful for understanding the physiological effect of intraocular pressure (IOP) on the accommodative mechanism. In this study, an IOP perfusion and recording system was developed and tested using 13 enucleated pig eyes. The vitreous chamber of the pig eyes was cannulated with a needle connected to two fluid reservoirs at different heights. One reservoir was set to achieve one of three baseline pressures of 5.5 mmHg, 13.0 mmHg and 20.5 mmHg. The other reservoir was moved to achieve pressures of 1.5 mmHg, 3.0 mmHg, 4.5 mmHg and 6.0 mmHg higher than the baseline pressure. The height differential between the reservoirs determined the amplitude of IOP changes. Rapid IOP changes were induced by switching the reservoirs with a solenoid pinch-valve. Two needles, one each attached to a pressure transducer were inserted into the anterior chamber and vitreous chamber respectively. Custom developed software was used to measure the anterior chamber pressure and vitreous chamber pressure at 80 Hz. A high-resolution continuous A-scan ultrasound biometer (CUB) was used to dynamically measure changes in ocular biometry including anterior chamber depth (ACD), lens thickness (LT) and vitreous chamber depth (VCD) while the vitreous chamber pressure was manipulated. The changes in ACD, LT and VCD were analyzed as a function of the pressure change. Perfusion-induced axial biometric changes were quantified by the slopes of linear regression relationships. Both anterior chamber pressure and vitreous chamber pressure changed relatively systematically with the induced vitreous chamber pressure changes (anterior chamber: $y = 0.863x + 0.030$, $r^2 = 0.983$; vitreous chamber: $y = 0.883x + 0.009$, $r^2 = 0.981$). At perfusion pressures of 5.5, 13.0 and 20.5 mmHg, the slopes for ACD were −5.72, −2.75 and −2.36 μm/mmHg, for LT were −3.31, −1.59 and −1.03 μm/mmHg and for VCD were 19.05, 8.63 and 5.18 μm/mmHg. The system was able to manipulate and monitor IOP while axial biometry changes were recorded. This system will allow the relationship between IOP and accommodation to be studied in non-human primate eyes.

1. Introduction

The physiological role of intraocular pressure (IOP) in the accommodative mechanism is still uncertain. The classical Helmholtz theory of accommodation describes the ciliary muscle contraction as releasing zonular tension around the lens equatorial edge to allow the lens to take on a more spherical shape (Helmholtz von, 1962). Fincham proposed that the change in shape of the crystalline lens was due to the elastic capsule molding the lens substance into an accommodated form (Fincham, 1937). These fundamental aspects of the accommodative mechanism have been verified (Glasser and Campbell, 1998, 1999; Glasser and Kaufman, 1999). An alternative theory of accommodation proposes that the zonular-lens diaphragm acts as a catenary and a pressure differential between the anterior chamber and vitreous chamber is produced by ciliary muscle contraction and this pressure differential acts on the catenary to cause the lens to take on a more spherical and accommodated shape (Coleman, 1970, 1986; Coleman and Fish, 2001). Evidence for this catenary theory comes from IOP recordings during ciliary muscle stimulation in primate eyes which show an increase in vitreous chamber pressure that was suggested to be due to ciliary muscle contraction accompanied by an initial decrease in anterior chamber pressure that was suggested to be due to facilitation of aqueous outflow (Coleman, 1986). The increased vitreous chamber pressure was suggested to prevent the posterior lens surface from moving backward during accommodation and

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the initial pressure differential was suggested to cause the lens to change shape to produce accommodation (Coleman, 1970, 1986; Coleman and Fish, 2001). This catenary theory emphasizes the role of IOP in the mechanism of accommodation and if true would challenge many current approaches to restore accommodation to presbyopic eyes since many of the approaches rely on the elasticity of the lens capsule (Glasser, 2006, 2008; Schor, 2009; Sheppard et al., 2010). Knowing how accommodation is affected by IOP can therefore help understand the mechanism of accommodation and evaluate if it is feasible to restore accommodation by means of IOP-induced lens forward shift as expected by the catenary theory (Heatley et al., 2004).

To understand the causal or consequential effects of a rapid IOP change on accommodation requires a system in which continuous and simultaneous measurement of IOP change and refractive or biometric accommodative change can be measured. To measure the pressure differential across the lens, both anterior chamber pressure and vitreous chamber pressure need to be recorded. To study the relationship between ocular biometric change and IOP, one of the two variables, either ocular biometry or IOP, would need to be manipulated so that the subsequent change in the other variable can be measured and studied. Enucleated pig eyes are frequently used to study physiology of IOP and aqueous outflow (Ruiz-Ederra et al., 2005; Wagner et al., 2004) as well as to understand lens biometry (Reilly et al., 2009; Vilupuru and Glasser, 2001; Wendt et al., 2011). Enucleated pig eyes lack ciliary muscle contraction and accommodation cannot be induced to produce ocular biometric changes, but pig eyes can still be used to evaluate ocular axial biometric changes caused by IOP changes. The system in this study was developed to manipulate IOP in addition to measuring IOP and ocular axial biometry. Since pig eyes are widely available and inexpensive, pig eyes were used for developing and testing a system to understand how ocular biometry changes with manipulation of IOP and so this system can ultimately be applied to study the effects of IOP changes on accommodation in monkeys.

A perfusion system including two fluid reservoirs was designed to induce a rapid IOP change in enucleated pig eyes while anterior chamber pressure, vitreous chamber pressure, and ocular axial biometry were recorded continuously and simultaneously.

2. Materials and methods

2.1. Materials

A total of thirteen pig eyes were used in six separate experimental sessions. For each experiment, four to six eyes from approximately 6 month old pigs were shipped overnight from a slaughter-house. The eyes were kept on ice in a bag filled with saline during transportation to the laboratory and refrigerated until used. Work on all eyes was started within 24 h of enucleation and remaining eyes were discarded. The conjunctiva, extraocular muscles and other extraocular tissues were removed. The optic nerve was cut off at the plane of the sclera. Each pig eye was glued into a custom-built eye holder with cyanoacrylate glue (Loctite 409; Henkel Corp., Westlake, OH) around the limbus of the eye (Fig. 1). The eye holder had a steel post screwed into it that was held by a clamp to hold the eye still throughout the experiment. The holder had a shape to approximately match the anterior sclera of a pig eye from the limbus to the equator and was open on the top and the bottom. The cornea protruded through the top opening with the eye facing upwards. The posterior 1/3 of the eye with the holder were bathed in saline so that the sclera was kept moist during the experiment. The eye holder had three holes for cannulation needles, one near the limbus and two posterior to the equator. The location of the holes ensured the cannulation needles entered the eye and the IOP was recorded and manipulated at similar locations for all 13 pig eyes.

2.2. Perfusion system

To induce a rapid vitreous pressure change, a perfusion system was developed. The system included two 1-L fluid reservoirs each with an internal diameter of 10 cm and each with an outlet tube at the bottom which passed through a two-way solenoid pinch-valve (075P3MP12-028; Bio-Chem Fluidics, Boonton, NJ) and thereafter converged, through a 'Y' adaptor, to a common outlet tube with a 16-G needle on the end. The reservoirs were filled with degassed saline and kept at different heights on two 80 cm long vertical posts each with a height scale in units of centimeters. The two reservoirs could

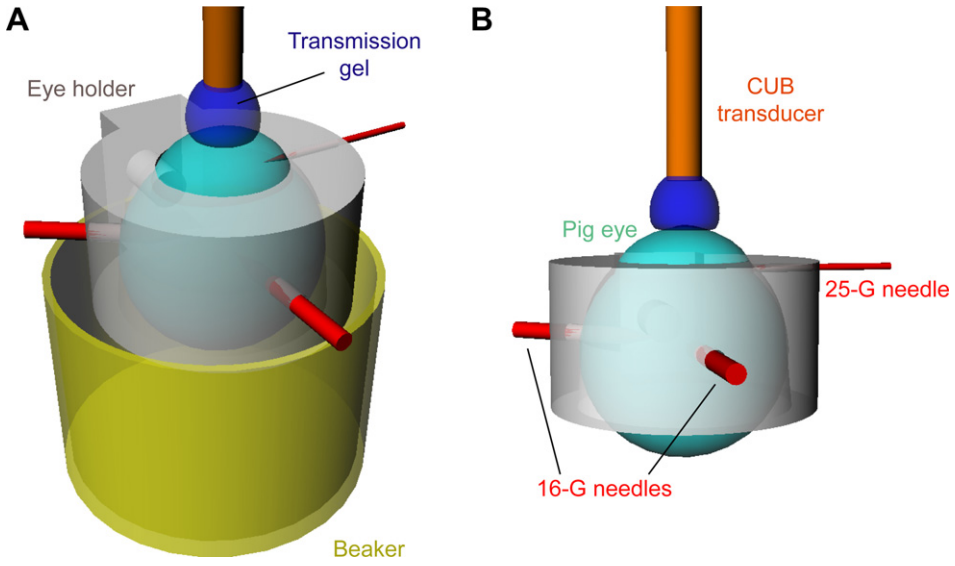

Fig. 1. Schematic diagram of the experimental setup. (A) The eye is oriented facing upwards and glued into a custom designed eye-holder with the cornea protruding through the upper opening and with the sclera bathed in saline in a glass beaker. The cornea has a bead of ultrasound transmission gel with the CUB A-scan transducer positioned in contact with the gel. Three needles are positioned in three different meridians. (B) A 25-G needle is in the anterior chamber and a 16-G needle is in the vitreous chamber, each independently connected to a pressure transducer (not shown) to measure the pressure. A second 16-G needle is inserted in the vitreous chamber to manipulate the IOP.

be independently raised and lowered to alter the perfusion pressure. The pinch-valve was controlled by computer via a digital-to-analog (D/A) converter (DI-148U, DATAQ Instruments Inc., Akron, OH) through a transistor–transistor logic (TTL) signal from a custom-built electronic circuit to switch the pinch-valve to pinch either one or the other input tube to close off either one of the saline reservoirs. The needle from the common outlet tube was inserted into the vitreous chamber of the pig eyes through one of the holes in the eye holder. Placing the reservoirs at different heights and switching between the reservoirs with the pinch-valve induced a rapid pressure change. This was used in preference to a system in which pressure changes were induced by moving the reservoir up or down (Ethier et al., 1993; Kee et al., 1997). To prevent blockage, a large diameter needle (16-G) was used and heparin (1 unit/ml; Hospira, Inc., Lake Forest, IL) was added to the saline in the reservoirs.

2.3. Anterior chamber and vitreous chamber pressure recording

Two USB port pressure transducers (PR41-X; Keller America, Inc., Newport News, VA) were used to simultaneously measure the anterior and vitreous chamber pressures respectively. The transducer for recording anterior chamber pressure was attached to a 25-G needle while the transducer for recording vitreous chamber pressure was attached to a 16-G needle (Fig. 1). The two needles were inserted through holes in the eye holder through the limbus into the anterior chamber and through the equatorial sclera into the vitreous chamber of the eye respectively. The needle in the anterior chamber was 13 mm higher than the needle in the vitreous chamber. The pressure transducers were mounted and maintained at the same heights as their respective needles by clamps. The needle for vitreous chamber pressure recording was at the same height in the eye as the needle for vitreous perfusion. The tips of these two needles were kept as close to each other as possible at the same level in the vitreous. Since on-axis A-scan ultrasound was later used (described below) to measure ocular biometric changes, the tips of needles were kept off-axis and close to the retina so as to be out of the ultrasound path.

A custom MATLAB program was developed to record anterior chamber pressure and vitreous chamber pressure and to control the pinch-valve to alter the perfusion pressure. The program sampled continuously from the two transducers at 80 Hz each, with a resolution of 0.00075 mmHg.

2.4. Ocular A-scan biometric measurement

A custom built continuous A-scan ultrasound biometer (CUB) (Baumeister et al., 2010; Beers and van der Heijde, 1996; Vilupuru and Glasser, 2005), with an acquisition frequency of 100 Hz, was used to dynamically measure anterior chamber depth (ACD), lens thickness (LT) and vitreous chamber depth (VCD). The CUB has a 10-MHz transducer with a 2 μm movement resolution (Beers and van der Heijde, 1994; Vilupuru and Glasser, 2005). To position the CUB transducer precisely, the transducer was clamped in a micromanipulator (D-10 Positioner; Research Instruments, London, UK) above the eye. The transducer tip was placed in contact with a drop of ultrasound transmission gel (Liquasonic Ultrasound Gel; Chester Laboratories Inc., Cincinnati, OH) on the cornea and oriented to give sharp A-scan peaks for all ocular surfaces. The CUB records the time between the peaks and the times were converted to actual distances by multiplication with accepted sound velocities of 1532 m/s for the aqueous and vitreous and 1641 m/s for the lens (van der Heijde and Weber, 1989; Vilupuru and Glasser, 2005). Sound velocities for the pig eyes have been reported to be almost identical to that of human eyes at room temperature (Thijssen et al., 1985) although the sound velocity of the lens can vary with age and is lower in the lens nucleus (Huang et al., 2007). The complete experimental setup is shown in Fig. 2.

2.5. Experimental procedure

Before each experiment, both pressure transducers were set to a “default zero” factory calibrated zero pressure prior to inserting the cannulation needles into the eye. The initial IOP measured in the enucleated pig eyes with both transducers was close to zero. The physiological pressure of pig eyes has been reported to be between 11 and 16 mmHg (Ruiz-Ederra et al., 2005), so baseline intraocular pressure recorded in the vitreous chamber was increased to 13.0 mmHg by adjusting the height of one of the opened saline reservoirs. The anterior chamber baseline pressure was also monitored and typically took less than 10 s to reach its equilibrium around 13.0 mmHg. In addition, two other baseline pressure levels, one 7.5 mmHg (10.2 cm H_2O) lower (i.e., at 5.5 mmHg) and the other 7.5 mmHg higher than 13.0 mmHg (i.e., at 20.5 mmHg) were also used to determine the effect of different baseline pressures. These three baseline pressure levels, low (5.5 mmHg), medium (13.0 mmHg) and high (20.5 mmHg) were used on all eyes. For each baseline starting pressure, four pressure increments each of 1.5 mmHg were sequentially applied. For example, at the medium baseline pressure, perfusion pressures were switched between $13.0 \leftrightarrow 14.5$, $13.0 \leftrightarrow 16$, $13.0 \leftrightarrow 17.5$, $13.0 \leftrightarrow 19$ mmHg, three times per trial. When switching from one baseline pressure to the next, i.e., $5.5 \leftrightarrow 13.0$ and $13.0 \leftrightarrow 20.5$ mmHg, step pressure increments of 7.5 mmHg were, therefore, also induced. The perfusion pressure was changed every 10 s by switching the pinch-valve, long enough for IOP to achieve an equilibrium state in the eye. Fig. 3 shows an example of how the perfusion pressure was altered first at one baseline pressure, and then the baseline pressure (medium) was switched to another (high). Throughout the experimental sessions, the two pressure transducers recorded the IOP continuously while the CUB measured the axial ultrasound biometry continuously.

2.6. Data analysis

For each pig eye, anterior chamber pressure, vitreous chamber pressure and axial biometry were recorded. When the pinch-valve was triggered to switch, to change the pressure, a time stamp was also recorded and logged into the IOP data file. Later during the data analysis, these time-stamps were located and the pressure differential was derived by subtracting anterior chamber pressure from vitreous chamber pressure. The five recorded parameters (anterior chamber pressure, vitreous chamber pressure, ACD, LT and VCD) were then averaged over the one second duration immediately before the switch and these values were considered as pre-pressure-increase or pre-pressure-decrease level. Similarly, post-pressure-increase or post-pressure-decrease levels of the five parameters were extracted and averaged over one second from the 9th to the 10th second after the switch, a time point when asymptotes had been reached. The difference between the pre-pressure and the post-pressure measurements was defined as the amplitude of the measured parameters. Positive amplitudes indicate an increase in pressure or biometry while negative amplitudes indicate decreases. For each intended pressure step, the amplitude of ACD, LT and VCD was averaged from all the pig eyes and was represented as “mean ± SEM”.

3. Results

The pig eye axial lengths were measured with the continuous ultrasound biometry and ranged from 20.61 mm to 23.94 mm (mean ± SD: 22.17 ± 1.07 mm). Since the axial lengths were similar,

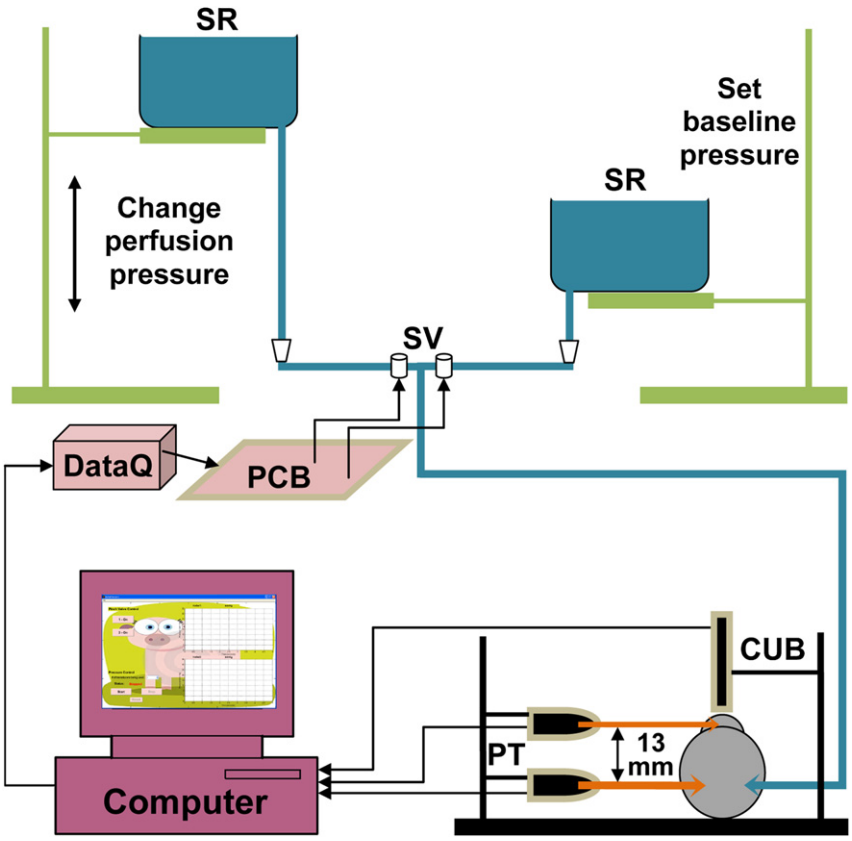

Fig. 2. Diagram of the experimental system. A TTL signal was sent by a computer to switch the pinch-valve. The solenoid pinch-valve switched between the two reservoirs to change perfusion pressure in the vitreous chamber of the pig eye. The computer recorded anterior chamber pressure and vitreous chamber pressure from two Keller pressure transducers and axial biometry from the CUB. SR: saline reservoir; SV: solenoid pinch-valve; PCB: printed circuit board; PT: pressure transducer; CUB: continuous ultrasound biometer.

it is believed that the vitreous chamber needles were in similar locations in all the eyes. In one of the thirteen eyes, the vitreous chamber pressure did not achieve an asymptote by 10 s after the pressure change while the anterior chamber pressure did, suggesting a partial blockage of the vitreous chamber pressure recording needle. This eye was excluded from further analysis. The intended pressure increments and decrements were recorded in the remaining twelve eyes. The largest pressure increment was not performed in one eye due to inadvertently omitting this final step

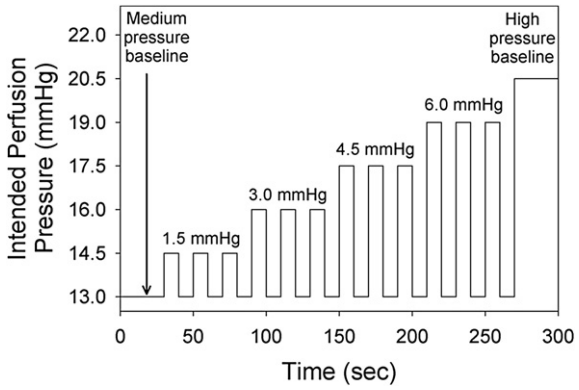

Fig. 3. An example of the sequence of altering perfusion pressure. The trial started from a medium pressure baseline level (13.0 mmHg). The perfusion pressure was raised and lowered three times each in different steps. Finally, the medium baseline pressure was raised to a high baseline pressure (20.5 mmHg). The interval for each pressure manipulation was 10 s.

in the experimental protocol. The data from this twelfth eye is included where appropriate. Fig. 4 shows the recorded changes in anterior chamber pressure (A & D), vitreous chamber pressure (B & E) and the pressure differential (vitreous chamber pressure minus anterior chamber pressure) (C & F) when the perfusion pressure was raised by 7.5 mmHg from low baseline pressure to medium baseline pressure (A, B & C) and from medium baseline pressure to high baseline pressure (D, E & F). For the eleven eyes shown, the pressure changes were reasonably consistent among the eyes.

Anterior chamber pressures changed in a slightly different way from the vitreous chamber pressures (Table 1). Over one second from the 9th to the 10th second after the pressure switch, the change in vitreous chamber pressure was significantly greater than the change in anterior chamber pressure for the intended pressure change, for example, from 5.5 to 13.0 mmHg the difference was 0.40 mmHg ($t = 3.39$, $p = 0.003$) and for the intended pressure change from 13.0 to 20.5 mmHg the difference was 0.28 mmHg ($t = 3.43$, $p = 0.003$). $T_{0.63\Delta P}$, the time to achieve 63% of the full response after the pinch-valve switch was used as a dynamic indicator of the pressure change. $T_{0.63\Delta P}$ was significantly faster for the vitreous chamber than for the anterior chamber, for example, from 5.5 to 13.0 mmHg the difference was 0.213 seconds ($t = 10.41$, $p < 0.001$), and for a pressure change from 13.0 to 20.5 mmHg the difference was 0.188 seconds ($t = 12.86$, $p < 0.001$). In addition, the vitreous chamber pressure tended to have an overshoot before reaching equilibrium as shown in Fig. 4B & E. Comparing the pressure changes between the two situations (5.5–13.0 mmHg versus 13.0–20.5 mmHg), the higher baseline pressure produced a faster pressure increase in both the anterior chamber

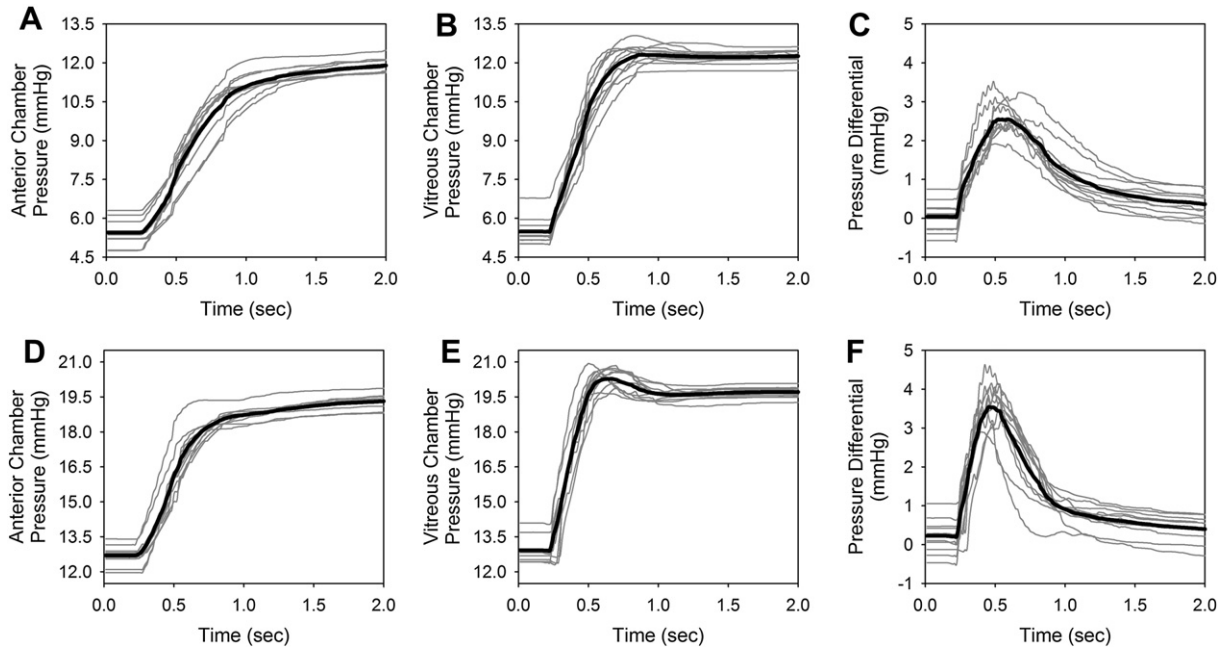

Fig. 4. Anterior chamber pressure (A), vitreous chamber pressure (B) and the recorded pressure differentials (vitreous chamber pressure minus anterior chamber pressure) (C) in response to an intended perfusion pressure change from 5.5 to 13.0 mmHg. Anterior chamber pressure (D), vitreous chamber pressure (E) and the pressure differentials (F) in response to an intended perfusion pressure change from 13.0 to 20.5 mmHg. The individual pressure changes from 11 pig eyes are shown as gray lines over 2 s and the black line represents the averaged pressure change of the recordings shown. The largest pressure increment was not performed in one eye. Another eye was excluded due to partial blockage of the vitreous chamber pressure recording needle.

(difference = 0.131 s, $t = 4.87$, $p = 0.001$) and the vitreous chamber (difference = 0.106 s, $t = 5.26$, $p < 0.001$) while the magnitude of the change in pressure was not significantly different between the two situations for the anterior chamber (difference = 0.14 mmHg, $t = 1.42$, $p = 0.185$) and the vitreous chamber (difference = 0.02 mmHg, $t = 0.33$, $p = 0.751$).

Fig. 5 shows the measured change in anterior chamber pressure, vitreous chamber pressure and pressure differential in 12 pig eyes as the perfusion pressure was increased and decreased by 1.5 mmHg, 3.0 mmHg, 4.5 mmHg and 6.0 mmHg at all three baseline levels. Measured pressure change was calculated based on the average pressure from the 1 s before the pinch-valve switch compared with the one second from the 9th to the 10th second after the switch. Anterior chamber pressure and vitreous chamber pressure changed systematically with the intended change in vitreous perfusion pressure (anterior: $y = 0.863x + 0.030$, $r^2 = 0.983$; vitreous: $y = 0.883x + 0.009$, $r^2 = 0.981$, differential: $y = 0.421x + 0.170$, $r^2 = 0.920$). The regression slopes are less than one for changes in both the anterior chamber pressure and vitreous chamber pressure, indicating that in general the intended pressure change was not achieved. The pressure differential between the anterior and vitreous chambers also depended on the intended perfusion pressure with a transfer ratio of 0.421.

The pressure-induced ocular A-scan biometric changes were measured and calculated in the current study from 12 pig eyes and grouped by different baseline perfusion pressures (Table 2). Fig. 6 shows that ACD and LT decreased and VCD increased with increased perfusion pressure. Lower baseline perfusion pressures tended to result in larger amplitude biometric changes. Linear regression was applied to the data from each row in Table 2. The regression slope indicates the rate of IOP-induced biometric change (Fig. 6). For baseline perfusion pressures of 5.5, 13.0 and 20.5 mmHg, the corresponding slopes were −5.72, −2.75 and −2.36 μm/mmHg for ACD, −3.31, −1.59 and −1.03 μm/mmHg for LT and 19.05, 8.63 and 5.18 μm/mmHg for VCD. VCD showed the largest rate of change while LT showed a slightly smaller rate of change than ACD.

Amplitude of the pressure differential was calculated by subtracting the initial pressure differential between the anterior and vitreous chamber (averaged pressure from 1 s before the pinch-valve switch) from the maximal or minimal pressure differential at between the 9th and 10th seconds after the pressure switch. Positive amplitude means the transient change in the pressure differential was higher than the initial pressure differential while negative amplitude means the transient change in the pressure differential was lower than the initial pressure differential. Fig. 7 shows that an increase in amplitude of pressure differential resulted in decreases in ACD and LT and an increase in VCD, however with considerable difference in slope between individual eyes due to inclusion of all three different baseline pressure levels. Fig. 6 and Table 2 show that baseline pressure affects the biometric changes. In addition, individual differences also exist as is evident in Fig. 7 as axial biometry of some eyes tended to change more than other eyes under the same differential pressure change.

Table 1
Intraocular pressure change (ΔP) and the duration between the pinch-valve switch to achieving 63% of the full pressure response ($T_{0.63\Delta P}$).

Intended ΔP (7.5 mmHg)	Location	Achieved ΔP (mmHg)		$T_{0.63\Delta P}$ (s)	
		Mean	SD	Mean	SD
5.5–13.0 mmHg	Anterior chamber	6.33	0.47	0.716	0.096
	Vitreous chamber	6.73	0.63	0.503	0.043
13.0–20.5 mmHg	Anterior chamber	6.47	0.33	0.585	0.067
	Vitreous chamber	6.75	0.53	0.397	0.049

4. Discussion

In this study, a pressure perfusion and recording system were developed and tested on enucleated pig eyes. The IOP was manipulated in two ways: 1) by keeping the eye at a constant

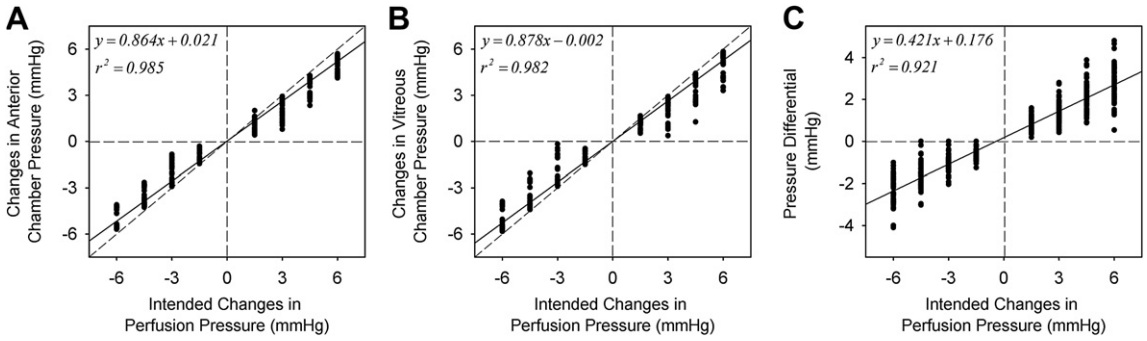

Fig. 5. Changes in anterior chamber pressure (A), vitreous chamber pressure (B) and pressure differential (C) as a function of intended changes in perfusion pressure in 12 pig eyes. One eye was excluded due to partial blockage of the vitreous chamber pressure recording needle. Negative perfusion pressure is a pressure decrease while positive pressure is a pressure increase. Linear regression lines are shown as solid lines and the dashed lines (A & B) are the 1:1 relationships.

pressure by connecting the eye to one of the fluid reservoirs and 2) by inducing a rapid pressure change by switching between two reservoirs with a pinch-valve. Switching between two reservoirs provides a faster pressure change than can be achieved by moving a reservoir up or down (Ethier et al., 1993; Kee et al., 1997). The posts to which the saline reservoirs were attached were marked every 2.0 cm to represent a 1.5 mmHg pressure change, based on 1:13.6 density relationship between water and mercury. The height of the reservoirs determined the intended perfusion pressure. The relatively large diameter of the reservoirs provided constant perfusion to the eyes. Although heparin was used to try to prevent vitreous blockage, blockages could still occur. If a partial blockage occurred in the needle used to record vitreous chamber pressure, measured vitreous chamber pressure would initially be lower than expected and would take longer to achieve its asymptote. This occurred in one of the thirteen pig eyes which was therefore excluded from further analysis. The slope of the relationships between intended and achieved pressures was less than one indicating that the intended pressure changes were not achieved. However, anterior and vitreous chamber pressures still continued to increase with increasing pressure steps, so this does not suggest blockage of the recording cannulas. It is unclear why the intended pressures were not achieved, especially for the vitreous chamber, in which the pressure should be transferred faithfully. It is unlikely this can be accounted for by inaccuracy in positioning the fluid reservoirs as the discrepancies tended to be towards lower pressures achieved than intended. It is also unlikely this can be because of leakage at the cannulation sites as the fluid reservoir volumes were sufficiently large to prevent this. The tubing compliance is not a cause of this problem either since pressure calibration with the reservoir, the transducer and the tubing showed the intended pressures were achieved over the range of pressures used in the experiments.

The catenary theory of accommodation emphasizes the role of IOP change in molding the lens into an accommodated shape (Coleman, 1970, 1986; Coleman and Fish, 2001). This theory suggests that an increase in vitreous chamber pressure supports the lens posterior surface to prevent the posterior lens surface from moving backward during accommodation (Coleman, 1970). Several studies have measured accommodative changes in ocular axial biometry in human subjects (Beauchamp and Mitchell, 1985; Bolz et al., 2007; Dubbelman et al., 2005; Ostrin et al., 2006) and in monkeys (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2005). All these studies show that the posterior lens surface moves backward during accommodation, and is not supported as suggested by the catenary theory. Although this undermines a fundamental aspect of the catenary theory, the relationship between accommodation and IOP is still not clear. Decreases in IOP with static voluntary accommodation have been recorded with tonometry in humans (Armaly and Jepson, 1962; Blake et al., 1995; Cassidy et al., 1998; Jenssen and Krohn, 2012; Mauger et al., 1984). One recent study used a dynamic contour tonometer to record IOP but dynamic changes in IOP with accommodation were not measured (Read et al., 2010). All these studies measured anterior chamber pressure and all showed a pressure reduction of 1–2 mmHg. A decrease in anterior chamber pressure has also been measured in humans when accommodative responses are stimulated with cholinergic agonists (Croft et al., 1996) or after right unilateral forced nostril breathing (Chen et al., 2004). A transient decrease in anterior chamber pressure is critical for producing the pressure differential that is central to the catenary theory (Coleman, 1970, 1986). However, static recordings of a decrease in anterior chamber pressure do not identify if this pressure reduction is transient or constant. A constant decrease in anterior chamber pressure would be incompatible with the catenary theory since it is a transient pressure differential that is suggested to be important for molding

Table 2
Changes in ocular biometrics, including anterior chamber depth (ACD), lens thickness (LT) and vitreous chamber depth (VCD) at different intended perfusion pressure levels.

Axial biometry	Baseline (mmHg)	Intended changes in perfusion pressure (mmHg)							
		−6.0	−4.5	−3.0	−1.5	1.5	3.0	4.5	6.0
ACD (μm)	5.5	33.7 ± 6.1	27.3 ± 4.8	19.8 ± 3.9	12.0 ± 2.3	−10.3 ± 2.2	−17.9 ± 3.8	−25.2 ± 4.7	−31.3 ± 6.0
	13.0	18.1 ± 2.3	13.7 ± 1.7	8.8 ± 1.3	5.5 ± 0.7	−2.6 ± 0.7	−6.4 ± 1.3	−11.2 ± 1.7	−15.5 ± 2.2
	20.5	15.9 ± 1.5	12.0 ± 1.2	7.9 ± 0.8	−4.6 ± 0.4	−1.6 ± 0.3	−5.0 ± 0.7	−9.2 ± 1.1	−13.3 ± 1.4
LT (μm)	5.5	20.1 ± 1.9	15.2 ± 1.6	10.8 ± 1.2	5.8 ± 0.6	−5.2 ± 0.6	−9.9 ± 1.2	−14.3 ± 1.5	−19.3 ± 1.9
	13.0	9.8 ± 0.9	7.8 ± 0.6	5.3 ± 0.4	3.4 ± 0.3	−2.6 ± 0.1	−4.2 ± 0.4	−6.9 ± 0.6	−8.7 ± 0.9
	20.5	2.6 ± 0.3	3.7 ± 0.4	5.0 ± 0.6	6.4 ± 0.9	−1.7 ± 0.1	−2.9 ± 0.4	−4.2 ± 0.6	−5.5 ± 0.9
VCD (μm)	5.5	−106.7 ± 6.6	−85.6 ± 5.0	−59.4 ± 4.6	−31.4 ± 2.7	34.6 ± 2.7	62.5 ± 5.1	90.2 ± 5.2	112.5 ± 6.7
	13.0	−50.2 ± 3.0	−38.3 ± 2.5	−23.5 ± 1.7	−10.5 ± 0.9	13.9 ± 0.8	26.4 ± 1.7	41.3 ± 2.6	53.4 ± 2.8
	20.5	−30.3 ± 5.6	−22.2 ± 5.6	−13.4 ± 4.3	−6.5 ± 1.2	8.6 ± 2.7	16.5 ± 4.0	24.5 ± 5.8	32.7 ± 1.5

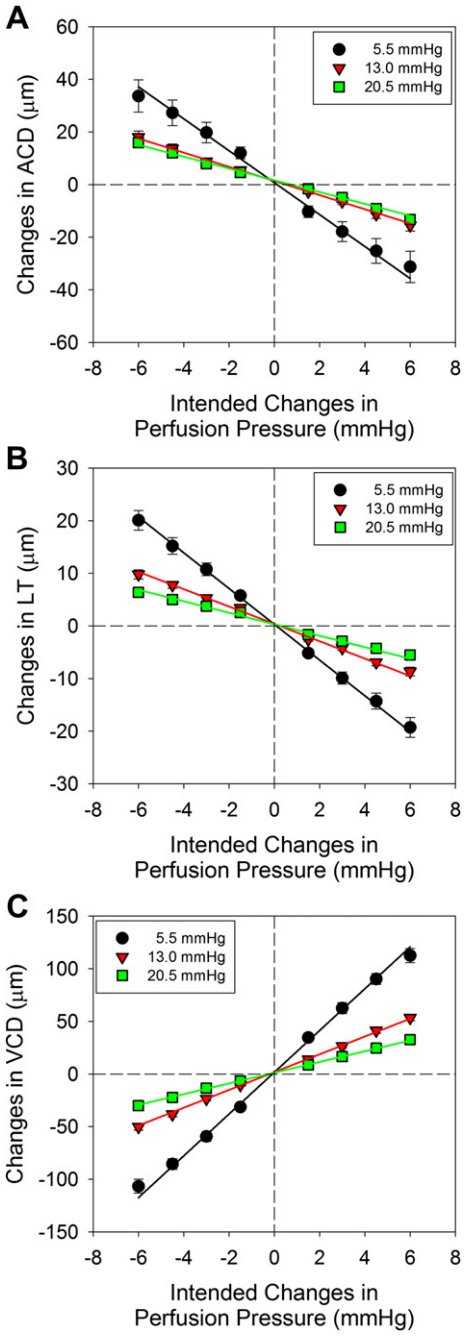

Fig. 6. Changes in ACD (A), LT (B) and VCD (C) as a function of intended changes in perfusion pressure in 12 pig eyes. Different symbols indicate three different baseline pressure levels, i.e., 5.5 mmHg (circles), 13.0 mmHg (triangles) and 20.5 mmHg (squares). Negative perfusion pressure is a pressure decrease while positive pressure is a pressure increase.

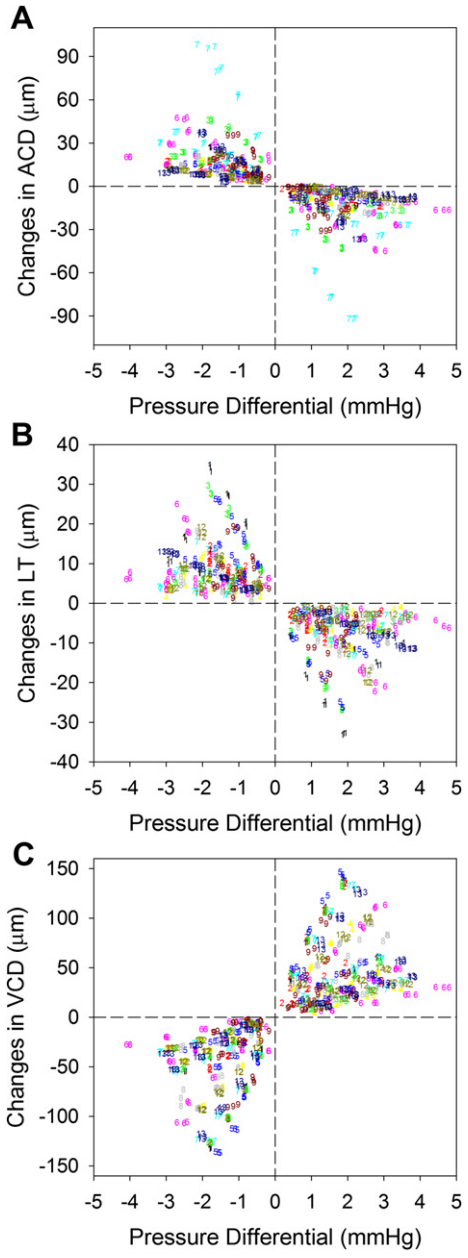

Fig. 7. Changes in ACD (A), LT (B) and VCD (C) as a function of the pressure differential amplitude in 12 pig eyes for all baseline pressure levels. A separate symbol number is used to represent each individual eye.

the lens into the accommodative form. Therefore, dynamic measurements of anterior chamber pressure are necessary for understanding the role of anterior chamber pressure in accommodation. Moreover, the association between the change in IOP and refraction is still unknown because none of the human studies measured refraction and IOP simultaneously. Axial length has been shown to increase after increasing IOP by applying an episcleral suction cup or by wearing swimming goggles and axial length has been shown to decrease and anterior chamber depth to increase after reducing IOP with oculopression (Leydolt et al., 2008; Read et al., 2011). However, to the knowledge of the authors, dynamic and simultaneous changes of ocular biometry and IOP have not previously been reported.

The induced pressure change in the pig eyes shows some similarities but also differences from the one pressure change shown that was recorded in a primate eye during stimulation of the ciliary muscle (Coleman, 1986). In pig eyes, switching to the higher perfusion reservoir resulted in pressure increase in both the vitreous and anterior chambers. In pig eyes, pressure differentials were produced with greater pressure changes in the vitreous than in the anterior chamber of up to 4 mmHg.

Fig. 8 shows a digitization of the graph showing measurement of anterior chamber and vitreous chamber pressure in a monkey eye during stimulation of the ciliary muscle (Coleman, 1986; Coleman and Fish, 2001). The units of "cm H_2O" in the original graphs

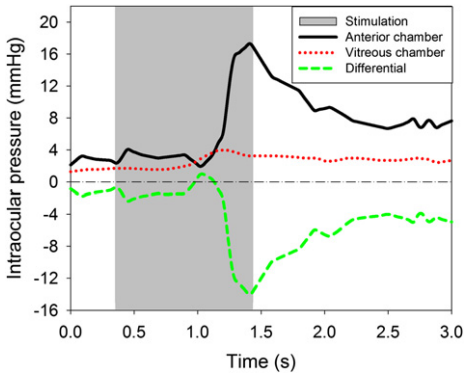

Fig. 8. Digitized reproduction of anterior chamber pressure (solid line) and vitreous chamber pressure (dotted line) from a prior study during stimulation of ciliary muscle in a primate eye (Coleman, 1986). The pressure differential (dashed line) shown in this figure was calculated by subtracting the digitized anterior chamber pressure values from the digitized vitreous chamber pressure values and not from simply digitizing the original pressure differential trace in the original published figure. The pressure differential calculated in this way differs from the pressure differential shown in the original figure for unknown reasons.

have been converted to "mmHg" in Fig. 8. As can be seen from the graph, vitreous chamber pressure (dotted line) increased about 600 ms after the electrical stimulation started and anterior chamber pressure (solid line) initially decreased by about 1.4 mmHg with a latency of about 600 ms. The latency of EW-stimulated accommodative refractive and biometric changes in monkeys is less than 100 ms (Vilupuru and Glasser, 2002, 2005). This suggests that the ciliary muscle stimulated accommodative response should have initiated roughly 500 ms before the recorded IOP changes. The initial anterior chamber pressure decrease and vitreous chamber pressure increase resulted in an initial pressure differential (dashed line) of about 2.3 mmHg. It is this pressure differential that was suggested to mold the lens into its accommodated state (Coleman, 1970, 1986; Coleman and Fish, 2001). However, the pressure differential starts much later than the stimulus, is considerably shorter in duration and is already over before the stimulus terminates. This argues against the pressure differential being the cause of the accommodative change in the lens. Obviously, to truly understand the relationship between the pressure changes and the accommodative response, both need to be measured continuously and dynamically as has been demonstrated in the present study in pig eyes. The pressure differential shown in Fig. 8 was calculated by subtracting the two digitized curves and shows an initial increase of 2.3 mmHg and later decrease of 15.0 mmHg. These are substantially larger than the early 0.5 mmHg and later 1.4 mmHg pressure differential shown in the original figure (converted from cm H_20) (Coleman, 1986; Coleman and Fish, 2001). The initial recorded decrease in anterior chamber pressure in the monkey eye is different from the perfusion-induced change in anterior chamber pressure in the pig eyes (Fig. 4). The initial decrease in anterior chamber pressure in the monkey eyes was suggested to result from a decrease in aqueous inflow and an increase in aqueous outflow due to the ciliary muscle contraction (Armaly, 1959; Coleman, 1986) which could not occur in the enucleated pig eyes. Therefore, although the current perfusion system may produce changes in vitreous chamber pressure that are similar to those recorded in the monkey eye, it is not able to reproduce similar changes in anterior chamber pressure as reported in the monkey eye during ciliary muscle stimulation. Never-the-less, the system described here can be used to obtain quantitative data to understand the relationship between IOP and accommodation in an animal model in which accommodation can be induced while recording and manipulating the IOP. That will be the subject of a future study.

The perfusion-induced vitreous chamber pressure increase in pig eyes led to a decrease in lens thickness which is opposite to the accommodative change predicted by the catenary theory. This experimental result is in accordance with finite element model simulation that also shows an increase in vitreous chamber pressure produces changes in the lens that are opposite to what is expected with accommodation (Martin et al., 2005). Although the absence of a ciliary muscle contraction in the enucleated pig eye means that the IOP changes do not necessarily simulate the pressure changes that might occur in a living monkey eye with accommodation, it is nevertheless important to know how the ocular axial biometry might change with direct manipulation of vitreous chamber pressure. It has been shown that voluntary accommodation in humans (Koeppl et al., 2005; Ostrin et al., 2006) and EW-stimulated accommodation in monkeys (Vilupuru and Glasser, 2005) result in an increase in LT and a decrease in both ACD and VCD. As can be seen in Figs. 6 and 7, the rapid increase in vitreous chamber pressure and resulting pressure differential in pig eyes caused a decrease in both LT and ACD and an increase in VCD. Besides the difference in induced pressure change in the current study compared to the prior study in monkey eyes (Coleman, 1986), the pig eye differs from the monkey eye in that the pig lens is thicker and more spherical (Glasser, 2003; Vilupuru and Glasser, 2001) and 6 month old pig lenses are stiffer than adolescent monkey lenses (Glasser, unpublished observation) and the pig eye is unlikely to undergo accommodative changes given these and other anatomical differences (Glasser, 2003). The likely scenario occurring in the pig eye with an increase in vitreous chamber pressure is as follows: an increased vitreous chamber pressure pushes on the posterior surface of the lens and pushes the lens towards the cornea causing the ACD to decrease, vitreous chamber depth to increase, and the anterior chamber pressure to increase. As the lens is pushed forward, the zonular fibers are passively stretched and tension of the zonular fibers increases due to this forward shift of the lens. Increased zonular tension likely then stretches the lens at its equator and results in the lens getting thinner as it is pushed forward.

In conclusion, the system implemented in the current study was able to manipulate IOP while simultaneously and continuously recording the IOP change and ocular biometric changes. This system will allow the relationship between IOP and accommodation to be studied in non-human primate eyes.

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