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Mechanical Stretching Forces Oppose Osmotic Lens Swelling

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In earlier work we demonstrated, with isolated lenses under *in vitro* conditions mimicking accommodation, that changes in lens volume occur in accord with the lens shape changes (Gerometta *et al.*, 2007; Zamudio *et al.*, 2008). *In vivo*, human lenses become rounder for closer vision and flatter for distant vision, a process involving a completely reversible change in shape. Our data indicated that lens volume was larger in the rounder, accommodative state than when the lens was in the flatter conformation (Gerometta *et al.*, 2007).

More recently, we determined that under hypo-osmotic challenge the lens not only changes volume but also its shape by becoming rounder (Kong *et al.*, 2009). In both the rounded accommodative state and the hypo-osmotic swelling conditions, the observed increase in lens “circularity” occurred because of an increase in the length of the axis between the anterior and posterior poles (A-P length). Osmotically induced lens swelling does not markedly affect the equatorial diameter (ED) indicating that the swollen lens exhibits a distinctive shape change. In the accommodative process, ED is increased as the lens is stretched, thereby shortening A-P length, with lens “circularity” restored by reversal of these changes.

These observations suggested to us that the mechanical stretching force that flattens the lens and shortens A-P length might oppose the force of osmotic swelling, which increases A-P length. In this short communication, we present data consistent with this idea.

Moreover, in principle, a defined hypotonicity might exist that would not elicit a net gain in lens volume when lenses are in the stretched conformation. This situation could occur if the stretching force were adequate to prevent the lens from adopting a more rounded shape, and if the capsule were sufficiently inelastic so that the lens cannot gain volume. Increases in lens volume can only occur if either the lens changes its shape, and/or increases its surface area. If the capsule were completely inelastic, volume increases would only occur by the lens becoming more spherical, a shape allowing maximal volume for a given surface area.

In practice, we also observed that tonicity-evoked changes in lens volume develop very gradually, and that relatively large tonicity shifts (i.e., ± 90 mOsm) are necessary in order to assuredly detect lens volume changes within a relatively short time frame (≈ 10 -20 min)

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(Kong *et al.*, 2009). Given these observations, we presently aimed to test the idea that the stretched, flattened lens might resist the swelling effect of a -90 mOsM, hypotonic solution.

Based on a lens topology similar to a torus, we developed a technique that allows volume determination from the lens cross-sectional area (CSA). The CSA was obtained from photographs taken perpendicularly to the lenticular A-P axis and computed with software. From the same digital images, we also measured the A-P length between the polar surfaces and the ED. This approach was described in detail in Gerometta *et al.*, (2007), and later used to determine rabbit and cow lens volumes with time of exposure to anisotonic conditions (Kong *et al.*, 2009). As discussed in the latter publication, our method enables measurements of rapid changes in lens volume, because sequential digital photographs can be captured quickly. A minor inconvenience is that the lens volume must be meticulously calculated from each of the captured images. However, our technique has a high resolution that readily enables detection of volume changes greater than 1% (Kong *et al.*, 2009), and importantly, the lens can be left untouched within a bathing chamber throughout the protocol.

In the present experiments, the lenses were bathed within the stretching apparatus that was described in detail for the rabbit lens (Zamudio *et al.*, 2008). The stretching device contained eight motors evenly mounted on a circular module. These motors were synchronized to simulate un-accommodation and accommodation by respectively transmitting radial stretching forces and relaxation to the lens as described in detail before (Zamudio *et al.*, 2008). The only difference was that the rabbit lens was now photographed perpendicularly to the A-P axis, as done earlier to calculate volume changes in the cow lens during accommodation (Gerometta *et al.*, 2007). Such lateral photographs of the rabbit lens captured both the length of the A-P axis and ED in the same image, which is a requisite of the volume calculation.

In a protocol done on 6 rabbit lenses, our approach entailed monitoring the lens volume under control conditions, then stretching it, exposing the stretched lens to a hypotonic medium for 20 min, followed by releasing the stretching force in the hypotonic solution. The measured lens parameters from these experiments are compiled in Table 1.

Because rabbit lenses do not stretch as well as lenses from higher primates, a small stretching force was applied until a significant reduction in A-P length could be observed (from 8.02 mm to 7.93 mm, Table 1). This degree of lens flattening caused the lenses to lose 6 μL (from 454 to 448 mm^3 , Table 1). After 10 min in 200 mOsM solution ($t=15$ min of the protocol), the flattened lenses gained 11 μL (from 448 to 459 mm^3). After an additional 10 min ($t=25$ min), the lenses gained only two more microliters, bringing lens volume to 461 mm^3 . At this point, the stretching force was released ($t=26$ min) causing the lenses to gain an additional 8 μL within 1 min, suggesting that the stretching force had impeded the inflow of fluid into the lenses by preventing them from becoming rounder.

Earlier we defined the ratio of the A-P length to ED as a measure of “circularity” and showed that this ratio increased significantly in rabbit lenses exposed to 200 mOsM solution for 20 min (Kong *et al.*, 2009). In contrast, in the present experiments, in which the 20 min of swelling occurred while the lenses were under a stretching tension, thereby inhibiting an

unimpeded increase in A-P length, the “circularity” ratio did not change significantly under the hypotonic conditions. This ratio remained at a value between 0.75 and 0.76 throughout the experimental protocol for the 6 lenses shown in Table 1, an observation also suggesting that the stretching tension had prevented the lenses from swelling freely.

Furthermore, if the 290-to-200 mOsm tonicity shift were independent of an opposing stretching force that impedes fluid uptake, then the changes in volume of lenses swollen in the stretched and relaxed conformations would be the same. Data in Table 2 suggest that the stretching force reduced the relative degree of swelling exhibited by the lenses. If stretching had no influence on the degree of lens swelling, the percentage changes in lens volume compared to the $t = 0$, control values should be similar for the two groups, but our results suggest otherwise.

Finally, our earlier data indicating that lens volume was larger in the rounder, accommodative state than when the lens was in the flatter conformation were consistent with a possible net flow of fluid to and from the lens during the accommodative process (Gerometta *et al.*, 2007). The present results are also in accord with net movements of fluid between the lens and the bathing medium in response to mechanical forces; i.e., stretching forces and hypo-osmotic forces have oppositely directed influences on lens volume. Putatively, stretching may inhibit the length of the A-P axis from freely increasing during swelling.

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Table 1

Effects of Hypotonic Conditions on the Anterior-Posterior (A-P) Length, Equatorial Diameter (ED), A-P to ED ratio, and Volume of Isolated Rabbit Lenses Held in a Stretched Conformation.

Time (min)	Lens Conformation	Bath Osmolality (mOsM)	A-P Length (mm)	ED (mm)	A-P/ED	Volume (mm ³)	Volume Change (mm ³)
0	Relaxed	290	8.02 ± 0.03	10.48 ± 0.03	0.764 ± 0.007	454 ± 49	-
5	Stretched	290	7.93 ± 0.03*	10.54 ± 0.03	0.751 ± 0.009*	448 ± 48*	-6
15	"	200	7.99 ± 0.03	10.62 ± 0.03	0.751 ± 0.013	459 ± 47*	+11
25	"	200	8.04 ± 0.03*	10.64 ± 0.02	0.754 ± 0.011	461 ± 48*	+2
26	Relaxed	200	8.10 ± 0.03*,**	10.61 ± 0.03**	0.762 ± 0.010	469 ± 51**	+8

Values are means ± SEM's from a set of 6 lenses treated identically. The isolated lenses were photographed from the side at the time intervals shown; pixel dimensions of the resulting images were converted into millimeters as described in Kong *et al.*, 2009. Lens volume was calculated as described in Gerometta *et al.*, 2007. Rabbit lenses were stretched in the chamber described in Zamudio *et al.*, 2008.

* Value significantly different than its respective antecedent (P< 0.05, as two-tailed paired data).

** Value significantly larger than its respective t= 0, control value (P< 0.05, as two-tailed paired data).

Table 2

Comparison of Control Rabbit Lens Volume to Volume after swelling for 20 min at 200 mOsM in the Stretched versus Relaxed Conformation.

Lenses in Stretched Conformation during Swelling				
Lens No.	Control Volume (mm ³)	Volume after 20 min at 200 mOsM (mm ³)	Change in Volume (mm ³)	Percentage Change in Volume
1	429	441	12	2.80
2	696	698	2	0.29
3	396	397	1	0.25
4	391	403	12	3.07
5	387	398	11	2.84
6	422	431	9	2.13
Mean:	454	461	7	1.90
SEM:	49	48	2	0.5
n:	6	6	6	6
Lenses in Relaxed Conformation during Swelling				
Lens No.	Control Volume (mm ³)	Volume after 20 min at 200 mOsM (mm ³)	Change in Volume (mm ³)	Percentage Change in Volume
17	366	373	7	1.91
20	362	393	31	8.56
21	347	366	19	5.48
22	351	367	16	4.56
23	355	368	13	3.66
24	352	362	10	2.84
29	350	361	11	3.14
30	337	350	13	3.86
Mean:	352	367	15*	4.25**
SEM:	3	4	3	0.7
n:	8	8	8	8

Data from lenses used in Table 1 are compiled for the individual values of lens volume changes for swelling in the stretched conformation. Rabbit lens volume changes for swelling in the relaxed conformation are values from Table 4 of Kong *et al.*, 2009.

* Volume change marginally higher in the relaxed conformation than in the stretched conformation with $P=0.065$, as unpaired, two-tailed data.

** Percentage change in volume significantly higher in the relaxed conformation than in the stretched conformation with $P<0.05$, as unpaired, two-tailed data.