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# Optic nerve head and intraocular pressure in the guinea pig eye

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ABSTRACT

The guinea pig is becoming an increasingly popular model for studying human myopia, which carries an increased risk of glaucoma. As a step towards understanding this association, this study sought to characterize the normal, developmental intraocular pressure (IOP) profiles, as well as the anatomy of the optic nerve head (ONH) and adjacent sclera of young guinea pigs. IOP was tracked in pigmented guinea pigs up to 3 months of age. One guinea pig was imaged *in vivo* with OCT and one with a fundus camera. The eyes of pigmented and albino guinea pigs (ages 2 months) were enucleated and sections from the posterior segment, including the ONH and surrounding sclera, processed for histological analyses - either hematoxylin and eosin (H&E) staining of paraffin embedded, sectioned tissue (n = 1), or cryostat sectioned tissue, processed for immunohistochemistry (n = 3), using primary antibodies against collagen types I–V, elastin, fibronectin and glial fibrillary acidic protein (GFAP). Transmission and scanning electron microscopy (TEM, SEM) studies of ONHs were also undertaken (n = 2 & 5 respectively). Mean IOPs ranged from 17.33 to 22.7 mmHg, increasing slightly across the age range studied, and the IOPs of individual animals also exhibited diurnal variations, peaking in the early morning (mean of 25.8, mmHg, ~9 am), and decreasing across the day. H&E-stained sections showed retinal ganglion cell axons organized into fascicles in the prelaminar and laminar region of the ONHs, with immunostained sections revealing collagen types I, III, IV and V, as well as elastin, GFAP and fibronectin in the ONHs. SEM revealed a well-defined lamina cribrosa (LC), with radially-oriented collagen beams. TEM revealed collagen fibrils surrounding non-myelinated nerve fiber bundles in the LC region, with myelination and decreased collagen posterior to the LC. The adjacent sclera comprised mainly crimped collagen fibers in a crisscross arrangement. Both the sclera and LC were qualitatively similar in structure in pigmented and albino guinea pigs. The well-organized, collagen-based LC of the guinea pig ONH is similar to that described for tree shrews and more similar to the human LC than that of other rodents that lack collagen. Based on these latter structural similarities the guinea pig would seem a promising model for investigating the relationship between myopia and glaucoma.



## 1. Introduction

The guinea pig has become a popular model for the study of human myopia (Howlett and McFadden, 2006, 2009), which is now epidemic in some populations (Vitale et al., 2009). Myopia, or nearsightedness, refers to the condition in which light rays from distant objects come to focus in front of the retina, resulting in blurred retinal images when this focusing error is not corrected. In most cases, the mismatch between the eye's length and its refracting power is a result of the eye being longer than normal. Importantly, the risks of potentially blinding pathological complications, including retinal detachment, macular degeneration and primary open angle glaucoma (POAG) are closely associated with the amount of myopia (Casson et al., 2007; Jonas and Budde, 2005; Liang et al.; Xu et al., 2007b). As the prevalence of high myopia, commonly defined as greater than -6 D, has also risen along with myopia prevalence overall, high myopia is expected to result in a higher number of associated pathologies (Pan et al., 2013; Pierro et al., 1992). In addition, uncorrected myopia is a leading cause of functional blindness (Pascolini and Mariotti, 2012).

*Abbreviations:* primary open angle glaucoma, POAG; lamina cribrosa, LC; optic nerve head, ONH; retinal ganglion cell, RGC; intraocular pressure, IOP; scanning electron microscopy, SEM; transmission electron microscopy, TEM; optical coherence tomography, OCT.

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In relation to POAG, a number of studies have reported increased risks for myopes, by two- to three-fold, with high myopes being more at risk (Jonas and Budde, 2005; Mitchell et al., 1999). In the Beaver Dam study, it was found that compared to emmetropes, myopes recorded higher IOPs and were also 60% more likely to have POAG (Wong et al., 2003). While glaucoma appears to be a multifactorial ocular disease, damage to the retinal ganglion cells (RGCs) and vision loss represent unifying end-points. Also of note in two recent studies, glaucomatous visual field damage and progression were found to be worse with increasing myopia (Perdicchi et al., 2007); optic nerve fiber loss was also more pronounced in highly myopic eyes than in less myopic eyes, implying greater susceptibility for optic nerve fiber damage in more myopic eyes, which also had larger optic discs (Jonas and Budde, 2005). Some of the evidence linking myopia with glaucoma and other sight-threatening pathologies is summarized in a recent review by Flitcroft (Flitcroft, 2012). However, note that some studies have reported no relationship between myopia and glaucoma progression in normal tension glaucoma (Sohn et al., 2010) and POAG (Hung et al., 2015).

In relation to nerve fiber loss in glaucoma, much attention has been paid to the lamina cribrosa (LC), which, in the human eye, is comprised of a collagenous meshwork spanning the scleral canal, deep in the optic nerve head, through which RGC axons pass to form the optic nerve. Changes in its structure have been strongly implicated in glaucomatous damage (Burgoyne et al., 2005; Quigley et al., 1983). Specifically, in glaucoma the LC is reported to deform, with the entire structure bowing posteriorly and its components compressed (Quigley et al., 1983; Yang et al., 2007). The deformation of the LC is postulated to generate shearing forces that disrupt critical transport processes within the axons of RGCs (Quigley et al., 1979; Sossi and Anderson, 1983). Other possible etiologies of axonal damage include impaired blood flow (Hayreh, 1969), laminar microarchitecture remodeling (Roberts et al., 2009), and biochemical alterations in extracellular matrix (Morrison et al., 1990). When IOP is artificially increased, the LC undergoes an anterior-posterior deformation, along with scleral canal expansion (Sigal et al., 2011), consistent with the suggestion that the biomechanical properties of the peripapillary sclera play an important role in the ONH changes in glaucoma (Girard et al., 2009a). Other glaucoma-related structural changes in the LC, specifically, increases in the area of laminar pores as well as shape changes (elongation) of the LC pores, have been observed in monkey eyes using advanced *in vivo* adaptive optics imaging (Vilupuru et al., 2007).

Of relevance to the current study, a previous study of enucleated highly myopic human eyes with and without glaucoma revealed the LCs of highly myopic eyes to be significantly thinner compared to those of less myopic eyes, and even thinner in highly myopic eyes with glaucoma (Jonas et al., 2004). The LCs of myopic eyes have also been reported to be more have more anterior surface irregularities (Miki et al., 2015). Either or both of these differences could contribute to the apparently greater susceptibility of myopes to glaucomatous optic disc changes (Fong et al., 1990; Scott and Grosvenor, 1993).

Of animal models used to study myopia, the chicken is one of the most widely used (Troilo et al., 1987; Wallman et al., 1987), yet it lacks a collagenous LC, and also lacks a concentration of astrocytic filaments in the retinal optic nerve junction (Morcos and Chan-Ling, 2000). Of animals currently being used for glaucoma research, well-organized collagenous LCs are present in the pig (Brooks et al., 1998), cat (Radius and Bade, 1982), dog (Brooks et al., 1989), and monkey (Gaasterland and Kupfer, 1974), but only one, the monkey, is also used as a myopia model.

While rodent models have been developed for many ocular diseases, including myopia and glaucoma (Faulkner et al., 2007; Gross et al., 2003), it is of interest that two of them, the mouse and rat, have non-collagenous LCs (Table 1) (Johansson, 1987; May and Lutjen-Drecoll, 2002). Astrocytes and oligodendrocytes represent the main non-neuronal, support component of the optic nerve head of rats and mice (Morcos and Chan-Ling, 2000). In a comprehensive study of OHN structures involving 18 different rodent species (Rodriguez-Ramos Fernandez and Dubielzig, 2013), the presence of an LC was confirmed in 4 species, including porcupine, capybara, flying squirrel and western gray squirrel, while the Norway rat and three species of mice were found to lack a LC. The lack of relevant histological sections of the optic nerve precluded evaluation of the LC for several species, including the guinea pig, which along with the mouse, also has application in myopia research. Nonetheless, several other studies have suggested that the guinea pig does have a LC, although its structure has not been studied in detail (Fujita et al., 2000; Furuta et al., 1993; Johansson, 1987). The study described here aimed to correct this deficiency – using a variety of microscopy techniques to fully characterize the ONH and adjacent sclera of nonmyopic guinea pig eyes, as well as to establish developmental intraocular pressure (IOP) profiles for the same.

## 2. Methods and methods

### 2.1. Animals

Pigmented and albino guinea pigs were obtained from Elm Hill Labs (MA, USA). Albino animals were included because of their availability, reports of refractive error abnormalities (Jiang et al., 2014; Wang et al., 2007), and use in other ocular studies (Pucker et al., 2014). Animals were maintained on a 12-h light/dark cycle and provided food and water ad libitum. For *in vivo* imaging, animals were anesthetized with ketamine (30 mg/ml) and xylazine (3 mg/ml). For *in vitro* studies of ocular tissue, animals were euthanized with an intracardial injection of sodium pentobarbital and eyes then enucleated and processed as described in detail below. The procedures used and numbers of animals used in each case are summarized in Table 2. Protocols were approved by the UC Berkeley and University of Houston Animal Care and Use Committees and conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

### 2.2. Intraocular pressure measurements

Pigmented guinea pigs ($n = 6$) underwent intraocular pressure (IOP) measurements on a weekly basis from ages 2–7 weeks, then monthly up to 3 months of age. Measurements were always taken at the same time of day (afternoon), to avoid any confounding effect of diurnal rhythms. Guinea pigs were awake and handheld for measurement, which made use of a rebound tonometer, following manufacturer instructions and set to the rat calibration (TonoLab, Colonial Medical Supply, NH, USA). At each time point, three sets of six measurements were recorded on the right and left eyes, and averaged for each eye.

Diurnal IOP rhythms were evaluated in 12 adult pigmented guinea pigs (age 12–18 months). Using a rebound tonometer as described above, IOPs were recorded from their right eyes at two-hour intervals across a 24-h period. Ten measurements were recorded at each time point and averaged. During the dark phase when lights were off (7:00 pm–7:00 am), measurements were made in the dark using a dim red LED headlamp.