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Association of Body Length with Ocular Parameters in Mice

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Abstract

Purpose—To determine the association between changes in body length with ocular refraction, corneal radii, axial length, and lens thickness in two different mouse strains.

Methods—Body length, ocular refraction, corneal radii, axial length, and lens thickness were measured for two inbred mouse strains: 129S1/SvJ (n=7) and C57BL/6J (n=10) from 4 to 12 weeks of age. Body length, from tip of nose to base of tail was obtained using a digital camera. Biometric parameters, corneal radii and refractions were measured using spectral-domain optical coherence tomography, automated keratometry and infrared photorefractometry, respectively. A mixed model ANOVA was performed to examine the changes in ocular parameters as a function of body length and strain in mice controlling for age, gender and weight over time.

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Results—C57BL/6J mice had significantly longer body length (average body length at 10 weeks, 8.60 ± 0.06 cm) compared 129S1/SvJ mice (8.31 ± 0.05 cm) during development ($p < 0.001$). C57BL/6J mice had significantly hyperopic refractions compared to 129S1/SvJ mice across age (mean refraction at 10 weeks, 129S1/SvJ: $+0.99 \pm 0.44$ D versus C57BL/6J: $+6.24 \pm 0.38$ D, $p < 0.001$). Corneal radius of curvature, axial length and lens thickness (except 10 weeks lens thickness) were similar between the two strains throughout the measurement. In the mixed model ANOVA, changes in body length showed an independent and significant association with the changes in refraction ($p = 0.002$) and corneal radii ($p = 0.016$) for each mouse strain. No significant association was found between the changes in axial length ($p = 0.925$) or lens thickness ($p = 0.973$) as a function of body length and strain.

Conclusions—Changes in body length are significantly associated with the changes in ocular refraction and corneal radii in different mouse strains. Future studies are needed to determine if the association between body length and ocular refraction are related to changes in corneal curvature in mice.

Keywords

body length; axial length; corneal radius of curvature; refractive error; myopia

Myopia is the most common refractive error, which is mainly caused by an excessive elongation of eye length during the critical period of visually-guided eye growth. Myopia affects around 41.5% of the adult population in the United States,¹ and greater than 80% of young children in several East Asian countries.^{2–5} The high costs associated with the optical correction of myopia, and the association between myopia and a number of vision threatening eye diseases (such as glaucoma, cataract, retinal tears and retinal detachment) make it a significant global public health and economic concern.⁶

Although a number of human studies have suggested both genetic and environmental influences on the development of myopia,^{7–10} the mechanisms underlying abnormal ocular elongation in myopic patients are unknown. In addition to genetic and environmental factors, human studies have also demonstrated a strong association between anthropometric dimensions (such as height and weight) and myopia in children.^{11–15} Generally, height has been found to be the closest correlate of eye size^{11–13, 16} with taller children exhibiting longer eyes and myopic refractions,¹² while heavier children showing a trend towards more hyperopic refractions.^{12, 13}

A large body of research conducted on various animal models including mammals,¹⁷ primates^{18, 19} and chickens^{20–22} have shown the importance of visual environment on normal development of the eye. One of the latest inclusions to this group of animal models is the mouse. In recent years, growing interest has developed in using the mouse model to study mechanisms of myopia since it offers the advantage of manipulating both genes and environment in the same animal using various genetic mutants.^{23–29}

Refractive development research with mice uses various transgenic mutants on different background strains to investigate mechanisms of visually-guided eye growth. There are known differences in both ocular (such as axial length,³⁰ biometric parameters,³¹ eye

weight,³² lens weight,³² retinal area³²) and physical (such as body weight)^{30, 32, 33} parameters between different background strains of mice. Therefore, normal genetic variations within species (such as differences in body weight or length)³⁴ could potentially influence the growth of the eye and different ocular components under different environmental conditions.³⁵ Previous mouse studies have reported an association between eye size and body weight. Zhou and Williams³² measured eye weight (as a measure of eye size), lens weight and area of the retina of 507 mice from 50 different strains. After correcting for age and sex differences using a multiple linear regression model, they found a positive correlation between body weight and eye size in mice. In another study, Chou et al³⁰ reported significant differences in regression slopes between the body weight and the intraocular pressure of C57BL/6J and DBA/2J mouse strains, accounting for differences in axial length observed between the two strains. By mapping the quantitative trait loci, a previous study has found body weight to be a significant predictor of eye size in an advanced intercross line of chickens.³⁶

Another important factor that could potentially influence ocular biometric parameters between different mouse strains is body length. Body length has previously been identified as an important determinant of eye size in other animal models (such as chickens).³⁶ However, no previous study has investigated the association between the changes in body length and axial length or ocular refraction in mice. In this study, we aimed to: 1) investigate the differences in refractive, ocular and anthropometric development of C57BL/6J and 129S1/SvJ mice and 2) examine the changes in ocular refraction, corneal radius of curvature and ocular biometric variables (axial length and crystalline lens thickness) as a function of body length and background strain for C57BL/6J and 129S1/SvJ mice during normal eye development.

METHODS

Subjects and Procedures

For eye and body length measurements, an in-house breeding colony with C57BL/6J (Jackson stock no: 000664, n=10, 7 male and 3 female) and 129S1/SvJ (Jackson stock no: 002448, n=7, 4 male and 3 female) mice (Jackson Laboratory, Bar Harbor, ME) was maintained at the Atlanta Department of Veterans Affairs Medical Center. Mice were kept in 12:12 hour light-dark cycles (~ 17 lux during the light phase) with mouse chow and water accessible ad libitum. All experiments were approved by the local Institutional Animal Care and Use Committee and adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Changes in refraction, corneal radius of curvature, axial length and other biometric parameters, and body length were measured every 2 weeks from 4 to 12 weeks of age for both inbred mouse strains.

Refractive error and ocular biometric measurements were obtained, as described previously.^{26, 33, 37} Eyes were first dilated with 1% tropicamide. Refractive error of each eye was measured with an automated photorefractor.²³ Refractions were first obtained with the mouse awake by gently restraining their tail in a dark room. Following the awake measurements, the mice were weighed on a digital weighing scale (Scalesonline, Cleveland, Ohio, USA). The mice were then anesthetized with ketamine and xylazine based on their

body weight (ketamine 80 mg/kg; xylazine 16 mg/kg). A second set of more stable refractive measurements were acquired under anesthesia with a standard deviation (SD) of less than 0.5 diopters (D).²⁶ Following refractive measurements, objective measurements of the anterior corneal radius of curvature were obtained using an automated keratometer.^{38, 39} The radii of curvature measurements from the keratometer are repeatable within a SD of ± 0.02 mm.³⁸

A 1310 nm spectral-domain optical coherence tomography (SD-OCT, Bioptigen Inc, Durham, NC, USA) device was used to measure axial length and ocular biometrics of the mouse eye, as previously described.³⁷ This SD-OCT provides reliable optical measurements of the mouse eye with an axial resolution of 8 μ m, and a standard deviation of 10 μ m on repeated measurements.³⁷ Axial length, distance between the anterior cornea to the retinal pigment epithelium (RPE) border was measured using the OCT calipers (calibrated with a refractive index of 1.433)⁴⁰ by an unmasked observer.

Finally, body length measurements in mice were acquired using a Kodak DC290 digital camera (Eastman Kodak Co., Rochester, NY, USA). Measurements were performed by holding the mouse vertically by their tail, against a ruler placed at 34 cm from the camera. To avoid variability between measurements, distance between the ruler and the camera was kept fixed for all images acquired during the study. One uncompressed, high resolution (75*75 dots per inch, DPI), standard RGB image was obtained for each mouse at each measurement session. Finally, at the end of the measurements, the mice were given yohimbine (2.1 mg/kg) to reverse the effects of anesthesia and to avoid the development of corneal lesions.⁴¹ The mice were kept warm on a heating pad until they recovered from anesthesia.

Data Analysis

After acquiring body length measurements, images were exported from the camera for further analysis. Body length was measured from the tip of the nose to the base of the tail (Figure 1A) using image analysis software ImageJ (National Institutes of Health, Bethesda, MD, USA) by an unmasked examiner. Previous studies have reliably measured body length in mice using similar methodology.^{42, 43}

Statistical analyses were performed using commercial software SigmaStat 3.5 (Aspire Software International, Ashburn, VA). Changes in refractive error, corneal radius of curvature, ocular biometric parameters, and body parameters (i.e. body length and body weight) between the two strains during the developmental period were analyzed with repeated-measures two-way analysis of variance (ANOVA) and Holm-Sidak post-hoc tests for statistical significance. A mixed model ANOVA analysis with 129S1/SvJ mice as control was performed using SAS (version 9.4, Cary, NC) to examine the changes in ocular refraction, corneal radius of curvature, axial length and crystalline lens thickness as a function of body length and strain controlling for age, gender and weight over time. Mixed model ANOVA analysis is frequently used to study linear relationship between an outcome variable Y (ocular refraction, axial length, lens thickness, and corneal radii) and a set of independent predictors X (body length and strain) through the expression $Y = Xb + e$, where b is the vector of regression parameters, and e is the random error or variability in the

outcome not accounted for by the predictors and their relationship with Y (in our case covariates age, weight and gender).^{44–46} The effect size of body length on each measured ocular variable was also calculated.⁴⁷ In addition, a linear regression analysis was carried out to examine the association between the changes in ocular refraction and ocular parameters of the mouse eye. A p-value of less than 0.05 was considered to be statistically significant. All data are expressed as mean $\pm$ standard error of mean (SEM).

RESULTS

Body Parameters

While both strains had similar body lengths at 4 weeks of age (average body length; C57BL/6J: 7.20 ± 0.11 cm, 129S1/SvJ: 7.22 ± 0.05 cm), C57BL/6J mice showed a significantly greater increase in body length from 6 weeks onwards through the rest of the developmental period compared to 129S1/SvJ mice (average body length at 10 weeks; C57BL/6J: 8.60 ± 0.06 cm, 129S1/SvJ: 8.31 ± 0.05 cm) (two-way repeated measures ANOVA age by strain interaction, $F(4,83) = 6.35$, $p < 0.001$, Figure 1A&B, Table 1).

Although the C57BL/6J animals appeared to weigh less during early ages of development, they were not significantly different from 129S1/SvJ animals at any measured time point of the study (two-way repeated measures ANOVA main effect of strain, $F(1,84) = 0.457$, $p = 0.509$, Figure 1C, Table 1). There was a significant increase in body weight for both strains from 4 to 12 weeks of age (two-way repeated measures ANOVA main effect of age, $F(4,84) = 307.41$, $p < 0.001$, Figure 1C).

Refraction and Corneal Radius of Curvature

There were significant differences in the profile of refractive development between the two strains across age (two-way repeated measures ANOVA age by strain interaction, $F(4,84) = 28.32$, $p < 0.001$, Figure 2A, Table 1). C57BL/6J mice had relatively hyperopic refractive errors at 4 weeks of age (mean refractive error, $+3.80 \pm 0.41$ diopters, D). The eyes became considerably more hyperopic to $+7.01 \pm 0.35$ D by 6 weeks, before stabilizing to ~ 5.5 D at around 8 weeks of age. In comparison, 129S1/SvJ animals were significantly myopic at early ages (mean refraction at 4 weeks, -4.92 ± 0.82 D), and remained relatively myopic throughout their development compared to C57BL/6J mice (mean refraction at 12 weeks, $+6.32 \pm 0.25$ vs $+1.45 \pm 0.39$ D for C57BL/6J and 129S1/SvJ mice, respectively, Holm-Sidak multiple comparisons at all ages, $p < 0.05$, Figure 2A, Table 1).

Corneal radius of curvature was not found to be different between the two mouse strains during normal visual development (mean change in corneal radii from 4 through 12 weeks of age, C57BL/6J: 0.15 ± 0.01 mm, 129S1/SvJ: 0.16 ± 0.002 mm, two-way repeated measures ANOVA main effect of strain, $F(1,83) = 0.52$, $p = 0.483$, Figure 2B, Table 1). Both C57BL/6J and 129S1/SvJ mice showed a significant flattening of the corneal radius of curvature with age (two-way repeated measures ANOVA main effect of age, $F(4,83) = 307.49$, $p < 0.001$).

Axial length and ocular biometrics

We examined the changes in axial length and other ocular biometric variables for both strains of mice. Axial lengths were not significantly different between the two strains at any age (mean axial length at 10 weeks, C57BL/6J: 3.24 ± 0.02 mm, 129S1/SvJ: 3.26 ± 0.02 mm, two-way repeated measures ANOVA main effect of strain, $F(1,83) = 2.37$, $p=0.145$, Figure 3A, Table 1). However, there was a significant increase in axial length for both C57BL/6J and 129S1/SvJ mice with age (two-way repeated measures ANOVA main effect of age, $F(4,83) = 252.26$, $p<0.001$).

As the crystalline lens is the most important refractive component in mice (about 50–60% of the mouse eye), we also examined age-related changes in the thickness of the crystalline lens for C57BL/6J and 129S1/SvJ animals. Both strains showed similar increase in lens thickness with age, except at 10 weeks' where 129S1/SvJ mice (2.02 ± 0.02 mm) had slightly thicker lenses (Holm-Sidak multiple comparisons, $p<0.05$) compared to C57BL/6J mice (1.99 ± 0.01 mm) (two-way repeated measures ANOVA age by strain interaction, $F(4,84) = 3.69$, $p=0.009$, Figure 3B).

Mixed model ANOVA analysis

In mixed model ANOVA, body length changes showed an independent and significant positive association with the changes in ocular refraction for each strain of mice (129S1/SvJ: $y = -40.64 + 5.18x$; C57BL/6J: $y = -40.64 + 29.71 + 2.36x$) controlling for age, gender, and weight over time ($F(1,60) = 10.08$, $p=0.002$, Table 2). No significant association was found between the changes in axial length ($F(1,60) = 0.01$, $p=0.925$) or crystalline lens thickness ($F(1,60) = 0.00$, $p=0.973$) as a function of body length and strain in rodents. Interestingly, after controlling for age, gender, and weight over time, changes in body length were found to be significantly associated with the changes in corneal radius of curvature for both strains of mice (129S1/SvJ: $y = 0.111 + 0.004x$; C57BL/6J: $y = 0.111 + 0.013 + 0.003x$, $F(1,59) = 6.2$, $p=0.016$, Table 2). In addition, a linear regression analysis revealed that the changes in ocular refraction were significantly correlated with the changes in corneal radius of curvature (slope 0.0004, $r = 0.28$, $p=0.01$) but not axial length (slope 0.0005, $r = 0.20$, $p=0.065$) in mice.

DISCUSSION

Our findings demonstrate that different background strains of mice show differences in the length of the body (measured from the tip of the nose to the base of the tail) and refractive error across development. After controlling for age, gender, and weight over time, the changes in body length were found to be significantly associated with the changes in ocular refraction and corneal radius of curvature, but not axial length in mice, suggesting that the relationship between body length and ocular refraction may be dependent on the changes in corneal curvature in murine eyes.

The mouse is a relatively new animal model in refractive development and myopia research. The mouse myopia model allows for probing potential gene/environment interactions in refractive development by altering visual environment (with lenses or form deprivation) in

transgenic mouse mutants with specific gene defects (see review).²⁷ Refractive development research with mice uses various knockout mutants on different background strains to investigate mechanisms of visually-guided eye growth. There are known variations in physical parameters (such as body weight)^{30, 32, 33} between different strains of mice, which could potentially influence the length and other biometric parameters of the eye in rodents.

We found significant differences in the body lengths of C57BL/6J and 129S1/SvJ mice. C57BL/6J mice had significantly longer body lengths compared to 129S1/SvJ mice from 6 to 12 weeks of age. While some previous mouse studies have reported significant differences in body weight between strains,^{30, 32, 33} this study reports differences in body length between two different strains of mice. Similar to our experiment, a previous study also found no significant differences in body weights of adult C57BL/6J mice and wild-type (WT) controls (*Vsx1*^{+/+}) of an OFF pathway mutant (*Vsx1*^{-/-}) on 129S1/SvJ background.³³ The body length and weight measurements from 4 through 12 weeks of age across both genders on these two strains are consistent with a large number of previously published data sets available at The Mouse Phenome Database (MPD) at the Jacksons Laboratory (<http://phenome.jax.org/>), Bar Harbor, Maine USA [MPD data sets: Tordoff2 (2001), Crabbe1 (2003), Pletcher1 (2007), Maurer1 (2009), Lightfoot1 (2010), Chesler3 (2011)].

Similar to our findings, a recent report by Chakraborty et al³³ found significant differences in the overall refractive error profile of C57BL/6J mice and *Vsx1*^{+/+} control mice on 129S1/SvJ background. The hyperopic refraction in our C57BL/6J mice increased until 6 weeks of age, at which it plateaued and remained relatively stable thereafter. This pattern was largely consistent with a number of previous reports on C57BL/6J strain.^{25, 26, 28, 38, 48–50} With regard to our 129S1/SvJ animals, a recent study³³ also found *Vsx1*^{+/+} controls on 129S1/SvJ background to be ~ 5 D myopic at 4 weeks, becoming mild to moderately hyperopic by 12 weeks of age.

We observed no significant differences in eye lengths of C57BL/6J and 129S1/SvJ mice across the developmental period, suggesting that the changes in axial length were not consistent with refractive changes observed for either strain. In agreement with our observation, a previous study³³ also found no significant differences in axial length normalized to the corresponding body weights of C57BL/6J and *Vsx1*^{+/+} control animals on 129S1/SvJ background. The age related increase in axial length for each strain was generally consistent with previously published data on these mice.^{31, 33, 38, 49, 51}

Our mixed model ANOVA analysis showed a significant and independent association of body length and ocular refraction in mice after controlling for age, gender and weight over time (estimated effect size: 0.43). The model revealed greater hyperopic refractions for C57BL/6J mice than for 129S1/SvJ mice, which is consistent with previous reports on these background strains.³³ For example, with an average body length of 8.60 and 8.31 cm at 10 weeks of age, the predicted refractions for C57BL/6J and 129S1/SvJ mice were calculated to be +9.37 D and +2.41 D respectively. As shown in our study, the importance of body length measurements increase even further when there are no apparent differences in body weight, a parameter previously used to minimize the influence of body size on eye size in mice.^{30, 33} Future mouse studies should account for variations in anthropometric variables (such as

body weight or height) for determining the ocular refraction, especially if the mouse genotypes are on different background strains.

In this study, changes in body length were not found to be associated with the changes in axial length (estimated effect size: 0.04). This is contrary to the general observation in chicken³⁶ and human studies.^{11–13, 16} These differences may occur due to differences in the definition of “body length” between studies (ex Prashar et al³⁶ measured body length from beak to ankle in chickens), experimental design or may represent species differences on the influence of body length on axial length. Alternatively, body length may be associated with the changes in axial length in other strains of mice not measured in this study. Future studies are warranted to determine the relationship between body length and axial length in other background strains of mice.

Body length was found to be significantly associated with corneal radius of curvature in mice (estimated effect size: 0.34). Consistent with our observation, body length was found not only to be a positive correlate of corneal radius of curvature in both chickens³⁶ and population based human studies,^{12, 13, 16} but an important determinant of both vertical and transverse diameters of the cornea during postnatal eye growth.^{52, 53} In the mixed model ANOVA, C57BL/6J mice with greater body length exhibited significantly flatter corneas (associated with more hyperopic refractions) compared to 129S1/SvJ mice with shorter body length. For instance, at 10 weeks, for a body length of 8.60 cm, the predicted corneal radius for C57BL/6J mice was found to be 1.5 mm compared to 1.4 mm for 129S1/SvJ mice with a body length of 8.31 cm. The association of changes in corneal radius of curvature with ocular refraction suggests that ocular refractions may be more dependent on corneal curvature than axial length in mice. In support of this, Zhou et al⁴⁹ reported a significant positive association between the changes in refractive error and corneal radius of curvature in C57BL/6J mice. A recent study by Bergen et al⁵⁴ also reported corneal contribution to ocular refraction in retina-specific tyrosine hydroxylase knockout mice where significant myopic refractions during normal visual development were found to be associated with increased corneal steepening.

Although the study provides important associations between ocular and physical parameters in C57BL/6J and 129S1/SvJ mice, it has some limitations. First, the study was performed on a relatively small sample size ($n = 7$ and 10 for each strain respectively). A greater sample size could have strengthened the correlation observed between body length and different ocular parameters. Second, the study was performed on inbred mouse strains. Although mouse studies frequently use inbred strains for research, these results should be tested on outbred strains for future use. Third, the mice were tested only during 4 through 12 weeks of refractive development, and the association between physical and ocular parameters may vary at ages outside the measurement period of the study. Finally, C57BL/6J and 129S1/SvJ mice used in the current study are two commonly used genetic background strains for creating various mouse genotypes employed in myopia research.^{25–27, 33, 55} However, myopia research also uses several other background strains of mice (such as C3H/HeJ, DBA/2J etc). Therefore, caution should be exercised in using these results for different mouse strains that were not measured in the current study.

In conclusion, different background strains of mice exhibit differences in body length. Changes in body length are significantly associated with the changes in ocular refraction and corneal radii, but not axial length in mice. Future studies are needed to determine if the association between body length and ocular refraction are related to the changes in corneal curvature in murine eyes. For myopia research that uses various transgenic mouse mutants on different background strains, it is essential to understand normal variations in physical parameters between different strains of mice to isolate a true phenotype from strain dependent genetic variations.

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Figure 1.

Body length (A, B) and body weight (C) measurements in mice. **(A)** Representative body length measurements of 10 week old C57BL/6J (left) and 129S1/SvJ (right) mice. Saline drops were applied to the eyes to keep them hydrated. **(B)** During the developmental period, C57BL/6J mice showed a significantly greater increase in body length from 6 through 12 weeks of age compared to 129S1/SvJ mice (two-way repeated measures ANOVA age by strain interaction, $F(4,83) = 6.35$, $p < 0.001$). Post-hoc comparisons at specific ages, $***p < 0.001$. **(C)** Despite significant increase in body weight with age (two-way repeated measures ANOVA main effect of age, $F(4,84) = 307.41$, $p < 0.001$), both mouse strains were found to have similar body weights throughout the developmental period (two-way repeated measures ANOVA main effect of strain, $F(1,84) = 0.457$, $p = 0.509$). Error bars represent standard error of mean.

Figure 2.

Refractive development in C57BL/6J and 129S1/SvJ mice. **(A)** Mice on C57BL/6J background strain showed significantly hyperopic refractions compared to mice on 129S1/SvJ strain throughout the refractive development measured from 4 through 12 weeks of age (two-way repeated measures ANOVA age by strain interaction, $F(4,84) = 28.32$, $p < 0.001$). Post-hoc comparisons are indicated by asterisks at each age: *** $p < 0.001$. **(B)** Significant flattening in corneal radius of curvature was observed for both C57BL/6J and 129S1/SvJ mice (two-way repeated measures ANOVA main effect of age, $F(4,83) = 307.49$, $p < 0.001$); however it was not found to be different between the two mouse strains during normal visual development (two-way repeated measures ANOVA main effect of strain, $F(1,83) = 0.52$, $p = 0.483$). Error bars represent standard error of mean.

Figure 3.

Axial length (A) and crystalline lens thickness (B) changes in mice. (A) Axial length showed a significant elongation with increasing age for both C57BL/6J and 129S1/SvJ mice (two-way repeated measures ANOVA main effect of age, $F(4,83) = 252.26$, $p<0.001$), but no significant differences between the two stains at any measurement age (two-way repeated measures ANOVA main effect of strain, $F(1,83) = 2.37$, $p=0.145$). (B) Except at 10 weeks' where 129S1/SvJ mice had slightly thicker lenses compared to C57BL/6J mice (two-way repeated measures ANOVA age by strain interaction, $F(4,84) = 3.69$, $p=0.009$), both strains showed similar increase in lens thickness with age. Post-hoc comparison indicated for 10 weeks with asterisks, $**p<0.01$. Error bars represent standard error of mean.

Table 1

Summary of mean changes in body length, body weight, ocular refraction, corneal radius of curvature, axial length and lens thickness from 4 through 12 weeks of age in inbred C57BL/6J (n=10) and 129S1/SvJ (n=7) mouse strains. All data are expressed as mean $\pm$ SEM. Significant p-values ($p < 0.05$) from repeated-measures two-way ANOVA investigating the main effects of age, strain and age by strain interaction are highlighted in bold.

Variables	Strains	Age (weeks)					p-value age	p-value strain	p-value age*strain interaction
		4	6	8	10	12			
Body length (cm)	C57BL/6J	7.20 $\pm$ 0.11	8.24 $\pm$ 0.10	8.40 $\pm$ 0.10	8.60 $\pm$ 0.06	8.65 $\pm$ 0.05	<0.001	0.054	<0.001
	129S1/SvJ	7.22 $\pm$ 0.05	7.91 $\pm$ 0.06	8.22 $\pm$ 0.06	8.31 $\pm$ 0.05	8.40 $\pm$ 0.06			
Body weight (gram)	C57BL/6J	13.20 $\pm$ 0.20	19.25 $\pm$ 0.47	20.75 $\pm$ 0.27	23.05 $\pm$ 0.42	23.80 $\pm$ 0.51	<0.001	0.509	0.260
	129S1/SvJ	15.14 $\pm$ 1.27	20.07 $\pm$ 1.27	21.93 $\pm$ 1.41	23.07 $\pm$ 1.76	24.07 $\pm$ 1.65			
Ocular refraction (diopters)	C57BL/6J	+3.80 $\pm$ 0.41	+7.01 $\pm$ 0.35	+5.35 $\pm$ 0.40	+6.24 $\pm$ 0.38	+6.32 $\pm$ 0.25	<0.001	<0.001	<0.001
	129S1/SvJ	-4.92 $\pm$ 0.82	-4.35 $\pm$ 0.39	-3.33 $\pm$ 0.61	+0.99 $\pm$ 0.44	+1.45 $\pm$ 0.39			
Corneal radius of curvature (mm)	C57BL/6J	1.32 $\pm$ 0.01	1.39 $\pm$ 0.01	1.43 $\pm$ 0.01	1.45 $\pm$ 0.01	1.47 $\pm$ 0.01	<0.001	0.483	0.497
	129S1/SvJ	1.32 $\pm$ 0.01	1.40 $\pm$ 0.001	1.44 $\pm$ 0.001	1.46 $\pm$ 0.01	1.48 $\pm$ 0.01			
Axial length (mm)	C57BL/6J	2.99 $\pm$ 0.01	3.12 $\pm$ 0.01	3.20 $\pm$ 0.01	3.24 $\pm$ 0.02	3.29 $\pm$ 0.01	<0.001	0.145	0.784
	129S1/SvJ	3.01 $\pm$ 0.03	3.14 $\pm$ 0.02	3.22 $\pm$ 0.02	3.26 $\pm$ 0.02	3.34 $\pm$ 0.02			
Lens thickness (mm)	C57BL/6J	1.69 $\pm$ 0.01	1.87 $\pm$ 0.01	1.94 $\pm$ 0.01	1.99 $\pm$ 0.01	2.04 $\pm$ 0.01	<0.001	0.262	0.009
	129S1/SvJ	1.71 $\pm$ 0.01	1.85 $\pm$ 0.002	1.94 $\pm$ 0.002	2.02 $\pm$ 0.003	2.06 $\pm$ 0.001			

Summary of estimated effect sizes of body length, strain and strain*body length interaction on various ocular parameters and respective p-values from the mixed model ANOVA examining the association between changes in ocular refraction, corneal radius of curvature, axial length and lens thickness as a function of body length and strain controlling for age, gender and weight over time. Significant p-values ($p < 0.05$) are highlighted in bold.

Table 2

Effect	Ocular parameters					
	Ocular refraction (D)		Corneal radius of curvature (mm)		Axial length (mm)	
	Effect size	p-value	Effect size	p-value	Effect size	p-value
Body length (cm)	0.43	0.002	0.34	0.016	0.04	0.925
Strain	0.79	0.0002	0.52	0.040	0.22	0.412
Body length*strain interaction	0.43	0.001	0.31	0.014	0.13	0.311
					0.04	0.752