

## The correlation between the intraocular pressure, central corneal thickness, and signalment of the horse

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### Abstract

The impact of central corneal thickness (CCT) on intraocular pressure (IOP) has been demonstrated in humans and various animal species but not yet in horses. The current study investigated the relationship between IOP and CCT in horses of different ages, sex, breed, and body weights using tonometry and pachymetry. Ninety-seven horses without ocular disease were examined at the Equine Clinic of the University of Veterinary Sciences Brno, Czech Republic, between 2019 and 2020. A complete ophthalmological examination was performed, including direct ophthalmoscopy and slit-lamp biomicroscopy, to include only horses with healthy eyes in the study. The central corneal thickness was measured with a pachymeter, and the IOP was measured with a tonometer. The effects of sex, age, breed, and horse weight on IOP and CCT were analysed. Measurements of IOP and CCT were acquired in all 97 horses. There was a significant correlation between CCT and age and weight in both eyes. Older and heavier individuals had high CCT values. Mares had lower IOP mean values compared to geldings and stallions. No correlation was found between IOP and CCT of the same eye. This study confirmed a relationship between individual variables such as age and weight on CCT, and sex on IOP. Based on this report, CCT should be considered a minor indicator when interpreting IOP values in healthy horses. Nevertheless, age and weight are essential constraints when interpreting CCT values and sex on IOP values in horses.

*Cornea, tonometry, equine ophthalmology, weight, pachymetry*

Applanation tonometry is the current method for measuring intraocular pressure (IOP), with the pressure being assessed from the force required to flatten the corneal apex according to the Imbert-Fick law (pressure = force/area) (Goldmann and Schmidt 1957; Stamper 2011; Aziz and Friedman 2018). Therefore, estimated IOP values depend upon corneal surface conditions such as the central corneal thickness (CCT) (Goldmann and Schmidt 1957). Additionally, a wide range of factors can affect IOP values, including patient positioning, head position, drugs, time of the day, and sex (Annear et al. 2012; Gelaw 2012; Allbaugh 2017). Almost all human studies have shown that CCT is positively correlated with IOP. The degree to which the CCT affects IOP varies between studies (Ekesten and Torrang 1995; Aghaian et al. 2004; Kotecha et al. 2009; Gelaw 2012; Ito et al. 2012). The impact of CCT in diagnosing glaucoma in humans is a current focus in ophthalmology; however, the effect of CCT on applanation tonometry was already discussed in the 1950s by Goldmann who recognized the CCT as a potential confounder to IOP measurement (Stamper 2011).

Intraocular pressure measurement has a significant role in early case detection and management of equine recurrent uveitis (ERU) and glaucoma (Annear et al. 2012; Thomasy and Lassaline 2015; Allbaugh 2017). Ocular hypertension is associated with an increased risk of developing glaucoma, and ocular hypotension can adversely impact the eye, including corneal decompensation, accelerated cataract formation, and discomfort (Schmack et al. 2010; Wada 2006; Thomasy and Lassaline 2015; Wang et al. 2019).

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Stabilizing the IOP has been shown to decrease the progressive loss of the field of vision. Accurate and precise measurement of IOP is, therefore, fundamental to managing ERU and glaucoma (Thomasy and Lassaline 2015; Gerding and Gilger 2016).

The CCT is determined *in vivo* using optical, digital, or ultrasonic pachymetry (Burdová et al. 2011; Ito et al. 2012; Thomasy and Lassaline 2015; Allbaugh 2017). In horses, CCT varies from 377  $\mu\text{m}$  to 657  $\mu\text{m}$  with a mean value of around 550  $\mu\text{m}$  (Van der Woerd et al. 1995; Ramsey et al. 1999; Featherstone and Heinrich 2013; Allbaugh 2017). Similar to IOP, CCT can vary between individuals due to weight (Ito et al. 2012), breed (Montiani-Ferreira et al. 2003), sex, and age (Gilger et al. 1991; Ofri et al. 1998a; Aghaian et al. 2004; Sanchis-Gimeno et al. 2004; Altinok et al. 2007; Burdová 2011; Ito et al. 2012; Kim et al. 2020; Büşra 2021), with the correlation of age being a matter of controversy in horses (Ramsey et al. 1999; Plummer et al. 2003; Thomasy and Lassaline 2015; Allbaugh 2017; Ansari 2019). Variations in CCT in different horse breeds have not yet been documented. An accurate determination of intraocular pressure (IOP) may need to be modified according to CCT to avoid pressure overestimation or underestimation, which is essential in the clinical evaluation of ophthalmic disease (Whiteacre et al. 1993; Ko et al. 2005, Gelaw 2012). Hence, this study aimed to determine the effect of CCT on IOP values obtained in horses and compare them according to the horse's signalment. Based on the proven impact of CCT in IOP in humans and dogs, we hypothesized that this relationship would also be observed in horses.

## Materials and Methods

### Case selection

Ninety-seven horses (194 eyes) aged 1–25 years with a weight of 85–760 kg were used in this study (Table 1). The CCT and IOP values for each study group are summarized in Table 1. The study population (female and male, castrated or sexually intact) consisted of 49.0% (n = 48) mares, 28.0% (n = 27) geldings, and 23.0% (n = 22) stallions. Breed data were available for all 97 horses, consisting of 54.2% (n = 52) Warmbloods, 19.8% (n = 19) American Quarter Horses, 14.6% (n = 14) Cold bloods, and 11.4% (n = 11) Thoroughbreds. All horses considered for the study were examined at the Equine Clinic of the University of Veterinary Sciences Brno between May 2019 and August 2020. For all privately-owned horses, owner consent was provided prior to study inclusion. Healthy animals without previous systemic or ocular disease were considered. Horses that needed sedation or auriculopalpebral nerve block were excluded from the study. The majority of horses were examined during a pre-purchase examination.

Table 1. Horse signalment with intraocular pressure (IOP) and central corneal thickness (CCT) of both eyes.

	n	Age (years)	Weight (kg)	IOP (mmHg)		CCT ( $\mu\text{m}$ )	
				LE	RE	LE	RE
Horses	97	9.8	518.9	26.1	26.5	816.2	810.9
Sex							
Gelding	27	10.9	533.9	26.9	27.4	815.8	815.2
Mare	48	11.4	520.8	25.6	25.7	824.9	817.9
Stallion	22	5.0	496.4	26.1	27.0	796.6	789.5
Breed							
Warmblood	53	10.5	564.3	25.3	25.7	825.4	817.8
AQH	19	7.1	421.4	26.3	27.0	792.9	793.5
Coldblood	14	9.9	537.9	26.3	26.1	806.3	804.9
Thoroughbreds	11	10.3	465.4	27.1	25.7	824.6	814.4

RE - right eye; LE - left eye; n – number; AQH - American Quarter Horse

### Ophthalmologic examination

All examinations and procedures took place between 08:00 h to 13:00 h. The horses were placed with their head above the heart in a normal head-carriage position. The adnexa, anterior, and posterior ocular segments of both

eyes were examined with a transilluminator, slit-lamp biomicroscopy (Reichert PSL Portable Slit Lamp, Reichert, Depew, USA), and direct ophthalmoscopy (direct ophthalmoscope Heine, BETA200, Gilching, Germany). Tonometry (Tono-Pen VetTM, Ametek, Reichert, Depew, USA) and pachymetry (PachPenR, ultrasound handheld pachymeter, Accutome INCR, Malvern, USA) were performed prior to the use of the mydriatic agent and the completion of the ophthalmological examination. After the data collection was completed, the pupils were pharmacologically dilated for at least 20 min, with a drop of mydriatic solution (Tropicamide 1% ophthalmic solution, Unimed Pharma, Bratislava, Slovakia). A veterinary ophthalmologist completed the ophthalmic examination (slit-lamp biomicroscopy, direct ophthalmoscopy) to assess the ocular fundus and the optic nerve disk. Following the ophthalmic examination, each patient was classified as healthy or having ocular disease (e.g., non-ulcerative keratitis, ERU, glaucoma). Patients with ocular disease were excluded from the study.

#### Tonometry and pachymetry

Before measuring IOP, all corneas were anaesthetised with a drop of topical anaesthetic solution (Oxybuprocaini hydrochloridum 0.4% ophthalmic solution, Unimed Pharma, Bratislava, Slovakia) applied to a randomly chosen eye (first tested eye was randomly selected). The IOP measurements were performed using applanation tonometry. A new Tonopen cover was used for each horse and Tonopen was calibrated before each horse. The operator touched the cornea with the pen tip until a reading was displayed. Only measurements with a standard error smaller than 5% were accepted. At least three consecutive IOP readings from each eye were obtained during tonometry. The average of these three values was considered the IOP for each eye. Following tonometry, nine consecutive readings were gathered with pachymetry (velocity set at 1,640 m/s) with standard deviations below 5%, taken at a single point on the cornea situated in the centre of the pupil of each eye. The tip of the pachymeter was disinfected with material provided by the manufacturer. The average of these values was considered the CCT for each eye. One examiner collected all information to lessen differences in readings attributable to the handler technique. After pachymetry, a fluorescein stain was performed to evaluate for iatrogenic corneal ulceration. All subsequent tonometry and pachymetry readings were obtained within 10 min of the ophthalmologic examination.

#### Statistical analysis

The influence of variables age, breed, sex and weight on IOP and CCT values were studied. The analysis for the left and right eye was performed separately. Statistical analysis was performed using multivariable General Linear Models (GLM) ANOVA design in statistical software for Windows version 6. Correlation between IOP and CCT of the left and right eye of each horse was performed by Pearson correlation coefficient. The level of significance was set at  $P < 0.05$ .

## Results

### Corneal thickness

There was no effect of sex or breed on CCT in both eyes; however, a significant correlation was found between increasing age and corneal thickness (both eyes  $P < 0.05$ , see Table 2, Figs 1 and 2). A positive relation was also discovered between weight and CCT in both eyes ( $P < 0.10$ , see Table 2).

### Intraocular pressure

There was no effect of age, weight, or breed on IOP by any eyes. Female horses had lower IOP values than males. No correlation was found between CCT and IOP by any of the eyes (Pearson correlation coefficient - right eye:  $r = 0.072$ ,  $P = 0.484$ ; left eye:  $r = 0.149$ ,  $P = 0.146$ ).

Table 2. The effect of age, weight, breed and sex on intraocular pressure (IOP) and central corneal thickness (CCT) values for each study group (GLM analysis). Significant effects are in bold.

	IOP LE		IOP RE		CCT LE		CCT RE	
	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Age	2.577	0.108	0.176	0.675	11.783	0.001	9.899	0.002
Weight	0.878	0.349	0.134	0.714	3.243	0.072	2.713	0.010
Breed	0.679	0.878	1.015	0.798	3.621	0.305	0.802	0.849
Sex	2.319	0.314	1.581	0.454	2.548	0.280	1.718	0.424

RE - right eye; LE - left eye;  $\chi^2$  - results of chi-square test; *P* - significance at  $< 0.05$

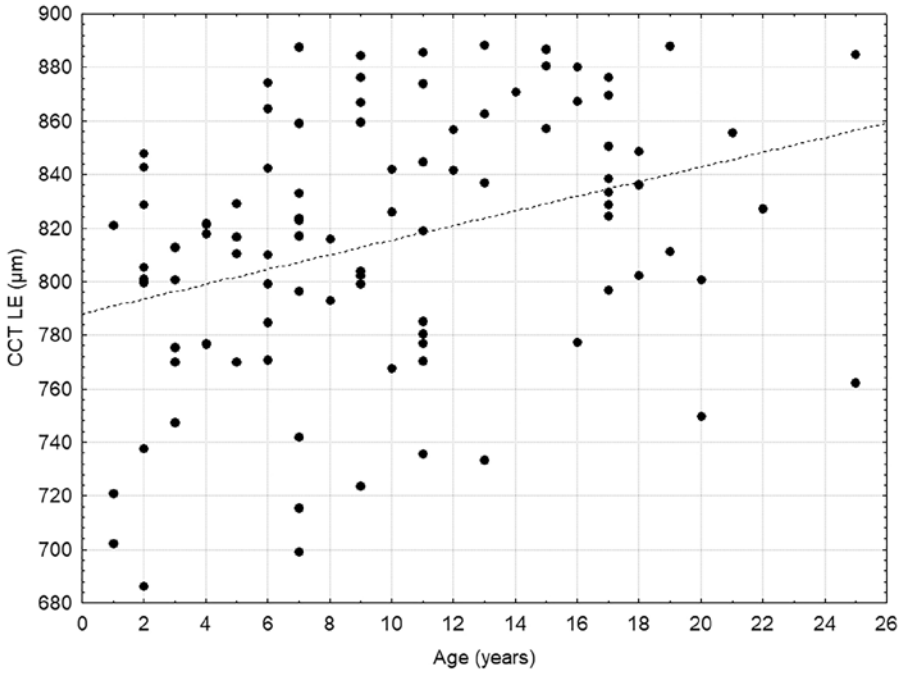


Fig. 1. The relation between age (in years, axis X) on central corneal thickness (CCT, in µm, axis Y) of the left eye (LE)

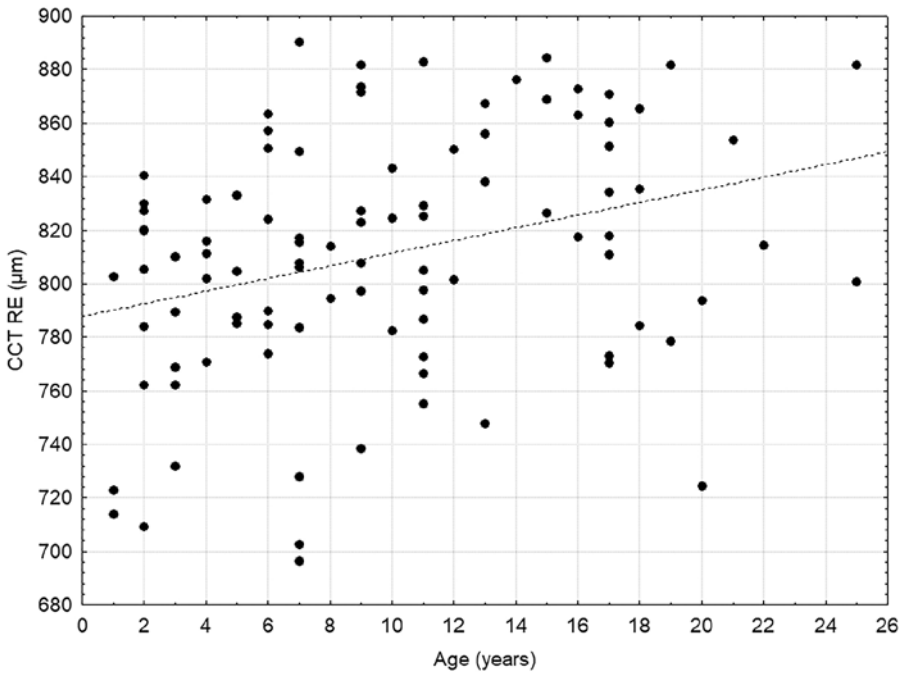


Fig. 2. The relation between age (in years, axis X) on central corneal thickness (CCT, in µm, axis Y) of the right eye (RE)

## Discussion

The accurate measurement of IOP and CCT is crucial in disease monitoring, progression, and assessing response to treatment in human and veterinary ophthalmology. However, determining an individual IOP value is more complicated as tonometry is influenced by the cornea's material properties, of which CCT is but one constituent (Whitacre et al. 1993). This finding should not be a surprise. Corneal pathology influences the relationship between IOP and CCT, so corneal diseases and surgeries that either increase or decrease the corneal thickness represent a challenge for IOP measurement (Wang et al. 2019). In equine medicine, pachymetry is less widely used, unlike tonometry. Pachymetry can have a supportive role in various clinical decisions, for instance, establishing patients' eligibility for refractive surgery, the diagnosis of the extent of corneal oedema, and providing essential references in the assessment of intraocular pressure in patients with glaucoma (Park et al. 2011; Stamper 2011; Ito et al. 2012). In our study, the CCT did not substantially impact the measurement of IOP in healthy eyes in horses ( $P = 1.00$ ), unlike humans and dogs documented in previous articles.

Effects of sexual hormones, like testosterone, on IOP, have been reported but without consistent findings in humans (Patel et al. 2018; Kim et al. 2020). A possible role for endogenous female hormones in the pathogenesis of OAG (open-angle glaucoma) cannot be excluded (Lee et al. 2003; Patel et al. 2018). Bilateral castration in male rabbits produced a decreased IOP (Hays et al. 2018). In a study on lions, the IOP was significantly higher in males than females (Ofri et al. 1998a). In our study, we detected that the mean IOP in mares (IOP = 25.6 mmHg [LE], IOP = 25.7 mmHg [RE]) was lower than in geldings (IOP = 26.9 mmHg [LE], IOP = 27.4 mmHg [RE]) and stallions (IOP = 26.1 mmHg [LE], IOP = 27.0 mmHg [RE]), however, the low statistical significance may be due to small population size.

Corneal epithelial and stromal thickness in children is significantly greater in boys than in girls (Kim et al. 2020). In adult humans, the effect of gender on CCT is contentious (Ekesten and Narfström 1992; Ekesten and Torrang 1995; Aghaian et al. 2004; Sanchis-Gimeno et al. 2004; Altinok et al. 2007; Kim et al. 2020). A possible correlation of CCT not only with gender but also with age has been suggested. Growth of the eye's gross structure could contribute to AHD changes in immature animals or humans (Pirie et al. 2014; Herbig and Eule 2015; Kim et al. 2020). In dogs, significantly thicker corneas have been reported in males than in females (Gilger et al. 1991; Park et al. 2011). According to the aforementioned literature, male species tend to have increased CCT. However, our findings did not indicate this, where no significant differences were documented for the CCT between all male and female horses. Our findings agree with other studies designed on horses (Ramsey et al. 1999; Ramsey et al. 2000; Plummer et al. 2003).

The correlation between CCT and horse age is controversial (Ramsey et al. 2000, Andrew et al. 2001; Ledbetter and Scarlett 2009; Hancox et al. 2002). Findings of the study in full-sized horses and miniature ponies (Plummer et al. 2003) did not identify an effect of age in IOP either in CCT. In the study reported here, a strong positive correlation of CCT with increasing age was found ( $P = 0.00059$  [LE],  $P = 0.00165$  [RE]) like in other studies (Ramsey et al. 1999; Ramsey et al. 2000; Andrew et al. 2001), where a positive correlation between corneal thickness and increasing age in certain breeds of horses was found. Ramsey et al. (1999) supported that 95% of corneal growing happens until the age of 6 months and is completed at the age of 6 years, whereas Plummer et al. 2003 found no correlation between age and corneal thickness in miniature horses; however, they supported that 95% of CCT development occurred until the age of 4 months.

Average IOP values vary among animal species (Ofri et al. 1998b) and humans of different races (Aghaian et al. 2004). Research on dog breeds has documented no difference in IOP (Gelatt and MacKay 1998). Our study found no IOP difference in horse breeds (25.3–27.1 mmHg). Few studies address factors influencing CCT in animals. Significantly thicker corneas have been reported in Labrador Retrievers than in a Beagle/Briard cross (Montiani-Ferreira et al. 2003). In human ophthalmology, racial differences have been widely studied, reporting CCT differences in some ethnic groups (Aghaian et al. 2004; Gelaw 2012). In our report, no differences were found in CCT values between breeds ( $P = 0.30$  [LE],  $P = 0.84$  [RE]).

Earlier reports suggested that IOP fluctuates with age. Various human studies have confirmed higher IOP in juvenile vs adult individuals, though some exhibited the opposite (Ekestén and Torrang 1995; Burdová et al. 2011; Ito et al. 2012). It has been assumed that relatively increased IOP in young animals may aid in promoting the growth of the globe (Ramsey et al. 2000). There was a positive correlation between age and CCT (the cornea gets thicker as the age increases, correlation  $R^2 = 0.2104$ ,  $P < 0.001$ ) in cattle (Büşra 2021). In the study reported here, a significant difference in values of IOP was not documented in adult and juvenile horses ( $P = 0.10$  [LE],  $P = 0.67$  [RE]); however, the number of juvenile horses was significantly smaller than that of mature ones (Table 1.).

In a recent study in human medicine, a higher body mass index (BMI) was associated with increased IOP and decreased IOP with weight loss (Ahn et al. 2020). These data support the idea that alterations in body weight affect intraocular pressures. In our study, a correlation of IOP with different body weights was documented but with low significance ( $P = 0.34$  [LE],  $P = 0.71$  [RE]); however, the IOP was not measured in alternations of the weight of a singular horse. Further research is needed to understand the relationship between body weight and IOP. This finding may also be helpful clinically. Variability in CCT in humans exists between individuals due to weight (Ito et al. 2012; Lam et al. 2017). Mean corneal thickness changed with weight ( $1.83 \pm 0.38 \mu\text{m/kg}$ ), and specifically, females had significantly thinner corneas ( $22.43 \pm 11.03 \mu\text{m/kg}$  than males) after adjusting for age and weight (Kim et al. 2020). Our study did not find a correlation between CCT and weight ( $P = 0.07$  [LE],  $P = 0.09$  [RE]). Further investigations involving a larger population of horses are needed to conclude whether there is a correlation between those two variables.

In conclusion, the current study revealed no correlation between IOP and CCT in the healthy eyes of horses. However, additional research is required to determine the effect of the CCT on IOP in diseased eyes to determine if the measurement of CCT and an adjustment of IOP is clinically relevant in horses. In addition, this study confirmed a strong positive correlation between CCT with age and a lesser one with weight. Also, female horses had lower IOP values than geldings and stallions. To the authors' knowledge, this is the first study where a correlation between weight and CCT is documented in horses.

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