Veterinary Ophthalmology (2007) **10**, 4, 211–215

Central corneal thickness in koi fish: effects of age, sex, body length, and corneal diameter

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Abstract

Objective To establish the central corneal thickness (CCT) of normal koi fish by ultrasonic pachymetry, and its relationship to age, sex, body length and corneal diameter.

Methods Age, sex and body length of 33 koi fish (17 male and 16 female fish) were recorded. Horizontal and vertical corneal diameters of each eye were obtained using Jameson calipers. Central corneal thickness of all eyes was measured by ultrasonic pachymetry. Intraocular pressure (IOP) by rebound tonometry was obtained for a subgroup of nine koi (18 eyes).

Results Mean central corneal thickness was 325.9 μm. Central corneal thickness of female koi was greater than CCT of male fish ($P < 0.01$). Central corneal thickness increased with increasing age overall and within both sexes ($P < 0.01$). Central corneal thickness increased with increasing body length ($P < 0.001$). For male and female fish, CCT increased with increasing horizontal and vertical corneal diameters ($P < 0.01$). Mean horizontal corneal diameter (HCD) was 8.05 mm, mean vertical corneal diameter (VCD) was 7.38 mm, and HCD was consistently greater than VCD. Mean IOP of a subgroup of these koi was 4.9 mmHg by rebound tonometry.

Conclusions Koi CCT increases with increasing age, body length and corneal diameter.

Key Words: corneal diameter, corneal thickness, fish, intraocular pressure, koi, pachymetry

INTRODUCTION

Ultrasonic pachymetry is an established method of obtaining *in vivo* corneal thickness measurements. It calculates corneal thickness measurements in micrometers based on the time it takes ultrasound waves to travel through the cornea at a precalculated speed (1640 m/s). Normal corneal thickness by ultrasonic pachymetry has been reported in humans and several veterinary species.[1–13] One study quantified corneal thickness in eight channel catfish.[14] The current report attempts to establish the central corneal thickness (CCT) of normal koi (*Cyprinus carpio*) by ultrasonic pachymetry, and its relationship to age, sex, body length and corneal diameter.

MATERIALS AND METHODS

Data collection

Sixteen female and 17 male koi fish of varying ages and color patterns were selected randomly from a privately owned collection. These koi were raised in 6-foot-deep fresh water ponds maintained with an automated filter system at 52–62°F. All fish were fed the same commercially available diet. The koi were selected from presorted gender-specific ponds and individually transferred to a small fresh water tub where sexing was confirmed. Gender and approximate age of each fish were recorded. An indelible ruler was present along the long edge of the rectangular tub and was used to measure body length (tip of nose to tip of tail) in cm, which was recorded for each fish (Fig. 1).

All further measurements were made while the koi were manually restrained by an experienced handler such that the body remained partially submerged within the water, and the eye elevated to near the air/water interface. No sedation or topical anesthetic was employed. Horizontal corneal diameter (HCD) and vertical corneal diameter (VCD) of each eye were measured using Jameson calipers[1] (Millenium Surgical, Haverford, PA, USA; Fig. 2) and recorded in mm. Central corneal thickness of each eye was measured in μm using a Tomey SP-3000 ultrasonic pachymeter (Tomey, Tokyo, Japan) set at a velocity of sound through the cornea of 1640 m/s. The probe tip was placed perpendicular to and lightly touching the central cornea for measurement (Fig. 3).

Figure 1. Orange-and-white koi fish held horizontally against a permanent ruler on the long side of a fresh water tub, for measurement of its nose-to-tail body length.

Figure 2. Jameson calipers being used to measure the corneal diameter of a koi fish.

Figure 3. Corneal thickness of a koi being obtained by careful placement of the ultrasonic pachymeter probe tip perpendicular to the central cornea.

This pachymeter is accurate to within ± 5 μm according to the manufacturer, obtains 10 CCT measurements each time, and automatically computes a mean and standard deviation. This mean CCT for each eye was recorded for analysis. Measurements with standard deviation greater than 5% were rejected. Additionally, intraocular pressure (IOP) measurements of 18 eyes of nine randomly selected koi were obtained using a rebound tonometer (Tono-Vet®, TiOLAToy, Finland) calibrated for use in dogs and cats. This instrument takes six measurements and internally averages them to determine the IOP measurement.

Data were collected for both the right and left eye of each koi, in a systematic manner. One author took all corneal diameter and tonometry measurements, and another made all corneal thickness measurements.

Statistical analysis

Repeated measures analysis of variance (RM-ANOVA) was used to analyze data from both eyes. Analysis of covariance (ANCOVA) was performed on the average values of the two eyes. As the populations of male and female koi varied significantly with respect to age, we attempted to control for this variable using statistical analysis, so that a comparison of CCT between the two gender populations could be made. To do this, we first tested for an interaction between sex and age effect on CCT, which was insignificant. This allowed us to then evaluate for the main effect of sex on CCT, while adjusting for age.

RESULTS

Sixteen female and 17 male koi were included in the study (Table 1). The average age of study participants was 2.8 years (range 1.5–6 years). Female fish (mean 3.16 years) were older than male fish (mean 2.47 years). Nose-to-tail length averaged 45.7 cm overall, and was greater for female (mean 53.0 cm) than for male fish (mean 38.9 cm). Using data from both eyes, average overall HCD was 8.05 mm, and was greater for female (mean 8.8 mm) than for male fish (mean 7.3 mm). Similarly, average overall VCD was 7.38 mm and was greater for female (mean 7.8 mm) than for male fish (mean 7 mm). Mean HCD (8.05 mm) was greater than mean VCD (7.38 mm), and this relationship also held true for both male and female fish. The ratio of HCD : body length was 1.76%. Mean IOP for both eyes of nine koi fish was 4.9 mmHg (range 1–11 mmHg).

Mean overall CCT was 325.9 μm. There was no significant difference in CCT between right and left eyes. Female fish had greater mean CCT (360.3 μm) than male fish (293.5 μm), and the overall sex effect when controlled for age was statistically significant ($P < 0.01$). Central corneal thickness increased with increasing age overall and within both sexes ($P < 0.01$). It also increased with increasing body length ($P < 0.001$). For male and female fish, CCT increased with increasing HCD and VCD ($P < 0.01$).

There was no significant difference between eyes in HCD or VCD. There was no significant difference between mean

Table 1. Summary of age, sex, body length of each koi, as well as horizontal corneal diameter, vertical corneal diameter, central corneal thickness, and intraocular pressure of each measured eye

Koi	Age (years)	Sex	Length (cm)	Eye	HCD (mm)	VCD	CCT (um)	IOP (mmHg)
F1	6	F	81.5	OD	14	12	350	
				OS	14	12	380	
F2	5	F	65	OD	10	8	350	
				OS	10	8	394	
F3	4	F	66	OD	10	9	455	
				OS	10	9	422	
F4	4	F	57	OD	9	8	353	
				OS	9	8	377	
F5	4	F	55	OD	6	5	287	
				OS	7	6	300	
F6	3.5	F	64	OD	10	9	420	
				OS	10	9	411	
F7	3	F	65	OD	8	7	420	
				OS	8	7	400	
F8	2	F	44	OD	9	7	343	
				OS	8	6	350	
F9	2	F	37	OD	6	6	390	
				OS	5	5	348	
F10	3	F	53	OD	9	9	432	
				OS	8	8	480	
F11	3	F	50	OD	10	10	287	
				OS	10	10	285	
F12	2	F	52	OD	8	8	368	
				OS	8	8	373	
F13	3	F	52	OD	10	9	380	
				OS	10	9	428	
F14	3	F	50	OD	11	9	388	
				OS	11	9	390	
F15	1.5	F	29	OD	6	4	230	11
				OS	6	4	252	8
F16	1.5	F	27	OD	6	5	256	8
				OS	6	5	230	5
M1	2	M	40	OD	7	6	327	
				OS	8	6	303	
M2	4	M	50	OD	9	8	366	
				OS	9	8	343	
M3	4	M	51	OD	10	10	352	
				OS	10	10	351	
M4	2	M	36	OD	6	6	300	
				OS	6	6	347	
M5	6	M	54.5	OD	10	9	401	
				OS	10	9	326	
M6	2	M	42	OD	8	8	304	
				OS	8	8	304	
M7	2	M	41	OD	7	7	257	3
				OS	7	7	271	3
M8	2	M	34	OD	6	6	276	1
				OS	6	6	277	1
M9	3	M	36	OD	6	6	240	4
				OS	6	6	283	2
M10	2	M	42	OD	9	8	253	2
				OS	8	9	263	4
M11	2	M	38	OD	7	6	258	
				OS	7	6	262	
M12	2	M	36.5	OD	7	7	285	
				OS	7	7	229	
M13	2	M	36	OD	8	8	330	
				OS	7	8	335	
M14	2	M	40	OD	5	6	341	
				OS	5	6	313	
M15	2	M	34	OD	7	7	266	3
				OS	7	7	274	4
M16	1.5	M	24.5	OD	6	5	205	5
				OS	6	5	214	8
M17	1.5	M	26	OD	7	6	258	8
				OS	7	6	264	9
Total	92.5		1509		531	487	21507	89
Average	2.8		45.73		8.05	7.38	325.86	4.94

HCD and mean VCD, but HCD was consistently greater than VCD within the same fish. This was true for both female ($P < 0.0001$) and male koi ($P < 0.05$). Neither HCD nor VCD was significantly affected by age or sex. Body length increased progressively with age over the age range included. Female koi grew more rapidly in length with age than male fish.

DISCUSSION

Mean CCT by ultrasonic pachymetry of koi fish is significantly less than that of CCT described in several mammalian species using the same technology. Corneal thickness of the normal human (548 μm),[15] dog (562 μm),[1] cat (569, 578, 755 μm),[4,5,8] alpaca (595 μm),[12] llama (608 μm),[12] miniature horse (785 μm),[9] and horse (893 μm)[10] all exceed 500 μm, compared to the koi fish's 326 μm. Such a magnitude of difference is important anatomic knowledge for purposes of corneal surgery. Additionally, the CCT of this population of koi fish was greater than that of 202 μm previously described for a small population of channel catfish, suggesting a species difference among fish.[14] This species difference may be explained, in part, by differences in size. The mean body length of that population of channel catfish was 39.8 cm, compared to the koi's 45.7 cm. At least within koi fish, CCT increases with body length, and this may hold true across species of fish. Additionally, canine CCT increases with increasing weight,[1] and body length and weight are expected to be positively correlated.

Koi CCT also increases with increasing HCD and VCD. In spite of their shorter body length, the catfish HCD (mean 12.1 mm) was much greater than the koi's (8.05 mm). As catfish CCT was less than koi CCT, a direct relationship cannot be made across the two fish species based on corneal diameter. We considered that HCD as a percentage of body length may make a useful cross-species comparison. However, catfish corneas had a greater HCD : body length ratio at 3.1% than koi at 1.76%. As koi have greater CCT than channel catfish, a direct relationship still cannot be made across the fish species. The positive relationship between CCT and HCD may still hold true within the catfish and other individual fish species. Within eyes, HCD consistently exceeds VCD in koi. This horizontally ovoid corneal shape is subtle when compared to the more pronounced horizontally elliptical shape identified in the horse, llama and alpaca.[9,12]

An increase in corneal thickness with age appears common. In the dog and cat, corneal thickness increases relatively rapidly during development, leveling off by adulthood around a year of age.[2,6] This is in conjunction with the period of developmental increase in body length and weight. Similarly, corneal thickness increases gradually in children, and adult values are reached at 5–9 years of age.[16] A more gradual increase in corneal thickness is reported to occur among older dogs in association with senile endothelial cell loss.[3] This population of koi was young compared to a long lifespan, which in well-cared for specimens can be similar to that of human beings. They were still in a rapid growth phase, which continues until about 8 years of age. It is reasonable to expect that koi CCT will continue to increase with increasing age and body length; however, these data are insufficient to prove this assertion. Obtaining pachymetry measurements on a wider age range of koi would have been useful, but older koi were unavailable for examination.

Of the above-mentioned domestic species in which CCT was measured by ultrasonic pachymetry, a gender difference in CCT was appreciated only in the dog but, unlike the koi, male dogs had thicker corneas than female dogs.[1] Men also had greater normal CCT than women in a recent large-scale study.[17] Gender differences can be partially explained by size differences, as male dogs and humans tend to outsize their female counterparts, while the opposite is true of koi. Female koi in our study outsized males on average (53 cm vs. 38.9 cm) and at all ages, and we did not control for body length in our analysis of sex effect on CCT.

Intraocular pressures of fish appear to be much lower than most domesticated mammals. A preliminary attempt to measure intraocular pressure by applanation tonometry[2] in this population of koi fish prior to the study period proved unsuccessful, as the majority of measurements were 'too low to measure'. The Tono-Pen Vet applanation tonometer (Medtronic Solan, Jacksonville, FL, USA) has a measurement range of 5 to 80 mmHg according to the manufacturer, suggesting that IOP of many of these fish was less than 5 mmHg according to that instrument. This was consistent with the inability of researchers to obtain IOP measurements in channel catfish with a Tono-Pen II® applanation tonometer.[14] We decided to attempt to measure IOP on a subgroup of fish using a rebound tonometer, the TonoVet®, which has recently been validated for use in domestic animal species.[18,19] Using this instrument, which can register IOPs as low as 0 mmHg, readings were relatively easy to obtain in the koi, and no topical anesthetic was needed. The mean IOP of 4.9 mmHg obtained here was much lower than that reported in normal domestic mammals by applanation tonometry.[20–24] It is also lower than the IOP reported by pressure transduction in normal zebrafish. There was significant variation by strain within zebrafish, but the strain means ranged from 10.8 to 20.6 mmHg, which is comparable to that of many mammals.[25] The IOP measurements we obtained may not, in reality, represent close approximations of actual IOP, as the Tono-Vet is calibrated for use in thicker mammalian corneas and has not been validated by comparison to direct transducer measurements in fish. A normal range of measurements for a given instrument can still provide useful information, providing the data are approximately linearly related to actual IOP and are repeatable. For instance, the Tono-Pen widely used by veterinary ophthalmologists is known to overestimate low IOPs in dogs and underestimate high ones.[26] The discrepancy in IOP measurements reported among fish suggests that cross-species assumptions about IOP should not be made among them. Normal fish of the same species should have tonometry performed using the same tonometer

for comparison whenever an attempt is made to establish a diagnosis of elevated IOP in fish for which there is no established normal IOP range.

ACKNOWLEDGMENTS

The authors wish to extend sincere thanks to Roland and Yoko de Aenlle of Toyoma Koi International, statistician David Schaeffer, and volunteers Sharon Burns and Jacqueline James, without each of whom this project would have been impossible. We gratefully accepted funding for statistical analysis from the Eye Care for Animals Associate Doctor Research Fund.

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