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● *Original Contribution*

VARIANCE OF SPEED OF SOUND AND CORRELATION WITH ACOUSTIC IMPEDANCE IN CANINE CORNEAS

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Abstract—The clinical standard for measuring corneal thickness is ultrasound pachymetry that assumes a constant speed of sound. The purpose of this study was to examine the variance of speed of sound and its relationship with acoustic impedance in healthy eyes of canines with a large age span. Corneal speed of sound and acoustic impedance were measured in 34 canine eyes at room temperature (21 ± 1°C). The mean speed of sound was 1577 ± 10 m/s ranging from 1553 to 1594 m/s. There was a strong correlation between speed of sound and acoustic impedance (R = 0.84, $p < 0.001$). Corneal speed of sound had a small variance in healthy canines over 1-year-old, but was significantly lower in younger canines suggesting an age effect. The strong correlation between corneal speed of sound and acoustic impedance may offer a potential means to noninvasively detect abnormal speed of sound for more accurate corneal thickness estimation. (E-mail: liu.314@osu.edu) © 2011 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Corneal speed of sound, Acoustic impedance, Density, Corneal thickness, Age, Canine.**

INTRODUCTION

Accurate measurement of central corneal thickness (CCT) has become clinically important because of its increasing role in glaucoma diagnosis and management. Many studies have documented a significant influence of CCT in the intraocular pressure (IOP) measurements using tonometric methods, where the measurement error could range from 0.2–1.4 mm Hg for each 20 μm deviation of CCT from the population mean (Ehlers et al. 1975; Whitacre et al. 1993; Stodtmeister 1998; Shah et al. 1999; Herndon 2006). More recently, CCT has been found an independent risk factor for glaucoma development (Gordon et al. 2002; Francis et al. 2008). According to a currently-adopted clinical model for predicting glaucoma risk, a 20 μm thinner CCT could increase the risk by three points (Medeiros et al. 2005), equivalent to an increase of IOP from 23 mm Hg to 26 mm Hg. This is clinically significant because even a 1 mm Hg increase in IOP was found to increase the hazard ratio for glaucoma progression by 11% (Bengtsson et al. 2007). These results emphasize the need for accurate determination of CCT.

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CCT is measured by either acoustic or optical methods. Ultrasound pachymetry is considered the clinical gold standard and measures CCT based on the assumed speed of sound. Similar assumptions about refractive index are made for optical methods. The use of a uniform speed of sound or refractive index is believed to generally apply in the population. Nevertheless, the extent of the variance and potential CCT measurement errors are not well-understood. If the inter-subject variance was large enough to introduce a CCT measurement error in the range of 10s of microns, it could have clinically significant implications to the risk profiling for glaucoma patients and suspects.

The variance of speed of sound in healthy human soft tissue was generally within ±5% (Goss et al. 1978). If a ±5% variance in speed of sound is present in the human corneas, it will translate into an error as large as ±25 microns in ultrasound pachymetry readings for a 500 μm thick cornea. Silverman et al. reported a range of 1600 m/s to 1616 m/s for the speed of sound in eight fresh, non-swollen bovine corneas. These eyes were collected from animals in a commercial abattoir whose age was likely similar because it was the typical practice of commercial abattoirs to euthanize animals at a certain age. In addition, the sample size ($n = 8$) was small. To our best knowledge, few studies have examined the variance of speed of sound in fresh normal corneas in animals of a large age span.

Since the 1960s, measurements of speed of sound have been carried out in postmortem dissected human corneas (Rivara and Sanna 1962; Tschewnenko 1965; Thijssen et al. 1985; de Korte et al. 1994; Ye et al. 1995). Most of the studies reported a mean speed of sound in the range of 1553 to 1575 m/s, with one study reporting a mean value of 1639 m/s (Tschewnenko 1965). Clinical instruments typically assume a speed of sound at 1640 m/s, which was motivated by achieving good agreement with optical methods (Waring et al. 1982). The lower speed of sound values measured in human corneal buttons may result from substantial corneal swelling due to the contact of the corneas with saline prior to or during acoustic measurements. Postmortem human corneas are known to rapidly absorb water due to the dysfunctional endothelia (Fischbarg and Maurice 2004). Silverman et al. examined the effect of hydration on speed of sound in bovine corneas and reported a decrease from 1605 m/s in non-swollen corneas to 1563 m/s in fully-swollen corneas when corneal thickness increased from 970 μm to 1579 μm in average (Silverman et al. 2009). In addition, it is often the case that a period of time (typically ranging from 6 to 48 h) has passed between the time of death and the time of eye recovery from human donors. Although the exact nature has not been determined, corneal tissue may have undergone some degradation during this period of time, which may explain the difference between the speed of sound used *in vivo* and measured *ex vivo*. Therefore, it is important to understand the population variance of speed of sound in non-swollen, fresh corneas immediately recovered after death. This accounted for one motivation for using canine eyes in the present study. Because of their availability immediately after euthanasia, the canine eyes can be measured at their natural, non-swollen, minimally degraded state.

Speed of sound is related to acoustic impedance in that acoustic impedance is the product of speed of sound and density. Theoretically, if corneal density varied little across subjects, speed of sound will be linearly correlated with acoustic impedance with the density being the slope. If corneal density differs significantly across subjects, the correlation will be altered or no longer exist. Su et al. reported a small variance of density directly measured in bovine corneas (Su et al. 2009). The animals were reported to be of similar age (about 2-years-old). Currently, there is lack of experimental data detailing the relationship between corneal speed of sound, acoustic impedance, and density in normal eyes of a large age span.

The objective of the present study is to examine the magnitude and variance of speed of sound in fresh, normal canine corneas. A secondary goal is to explore the relationship between corneal speed of sound and acoustic impedance. Canine corneas were used in this study not only because of their availability within a short postmortem time from animals of a large age span but also because of their similarity to human corneas. According to our initial tests and reported data (Elsheikh et al. 2008), the thickness and biomechanical properties (*i.e.*, modulus) of canine corneas are close to those of human's, making it a good animal model prior to extensive studies in less available human donor eyes.

MATERIALS AND METHODS

Sample preparation

Thirty-four fresh canine globes were collected immediately after euthanasia from healthy dogs that were humanely euthanized for population control purposes at a local animal shelter. The use of the biologic specimens from the animal shelter was approved by the university's Institutional Animal Care and Use Committee (IACUC). Age was recorded based on birthdates or estimated by experienced veterinarians based on anatomic information such as that of teeth for those without birth records. The animals were categorized into three age groups: puppy (below 1-year-old, six dogs), young (1- to 5-years-old, four dogs) and old (above 5-years-old, four dogs). Three dogs in this study were undetermined in age and not included in the analyses related to age effects.

Corneal epithelia were carefully removed using a surgical blade before all measurements to reduce the effect of potentially degrading epithelial integrity over time commonly seen in postmortem eyes. This manipulation was feasible according to our preliminary tests that showed minimal alterations in the acoustic reflections at the ultrasound frequencies used in this study comparing the signals from corneas initially with intact epithelium and then after epithelial removal. CCT was first measured in the globes using an ultrasound pachymeter (DGH-550 PACHETTE 2; DGH Technology, Inc., Exton, PA, USA). Three measurements were recorded and the average was used for further analysis. All measurements were completed within 2 h postmortem at room temperature (21 ± 1°C).

Acoustic impedance measurement

Corneal acoustic impedance was first measured in the intact globe, while maintaining an IOP around 15 mm Hg using a saline column connected to the vitreous chamber through a needle (Fig. 1). The globe was immersed briefly in a saline bath during the acoustic measurements. An unfocused ultrasound transducer (XMS-310; Panametrics-NDT, Waltham, MA, USA) with an element size of 2 mm was used. The nominal