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Doppler ultrasonography of the *pectinis oculi* artery in harpy eagles (*Harpia harpyja*)

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Abstract

Twenty harpy eagles (*Harpia harpyja*) without systemic or ocular diseases were examined to measure blood velocity parameters of the *pectinis oculi* artery using Doppler ultrasonography. Pectinate artery resistive index (RI) and pulsatility index (PI) were investigated using ocular Doppler ultrasonography. The mean RI and PI values across all eyes were 0.44 ± 0.10 and 0.62 ± 0.20 respectively. Low RI and PI values found in the harpy eagle's *pectinis oculi* artery compared with the American pekin ducks one and other tissue suggest indeed a high metabolic activity in *pecten oculi* and corroborates the hypothesis of a nutritional function and/or intraocular pressure regulation.

Keywords: Avian posterior segment, Pulsatility index, Raptors, Resistive index.

Introduction

The harpy eagle (*Harpia harpyja*) is the largest and most powerful neotropical raptor. They primarily inhabit the Amazon rainforest, but can be found extending from Mexico to South of Brazil (Banhos *et al.*, 2016). They are considered a vulnerable species according to the Brazilian list of endangered fauna (Brazil, 2014) due to deforestation, given their dependency on rainforest habitat, where they builds nests within the canopy and hunts tree-dwelling mammals like sloths (Banhos *et al.*, 2016). As are all eagles, they are primarily visual hunters, with eyes that contain many adaptations for increased visual acuity, including large globes (Güntürkün, 2000; Jones *et al.*, 2007), accommodation by corneal and lenticular measures (Samuelson, 2007), bifoveate retinae (Tucker, 2000), and an anangiotic (avascular) retina (Ruggeri *et al.*, 2010).

As with all avian species, an anangiotic retina leaves the inner retinal layers without a direct blood supply, therefore limiting delivery of nutrients and rapid removal of wastes. However, a vascular structure called the *pecten oculi* is found projecting anteriorly from the optic disc into the vitreous body of all birds (class Aves or clade Avialae) (Brach, 1977; Kern, 2006; Kiama *et al.*, 2006; Rahman *et al.*, 2010; Micali *et al.*, 2012; Mustafa and Ozaydjn, 2013). *Pecten oculi* are pigmented, vascularized, and are traditionally classified into one of three morphologies: 1) conical

(e.g. as found in kiwis); 2) vaned (e.g. as found in ostriches, rheas, and tinamous), or 3) pleated (e.g. as found in all the other avian species) (Brach, 1977; Baumel, 1993; Montiani-Ferreira, 2001; Kern, 2006; Kiama *et al.*, 2006; Rahman *et al.*, 2010; Micali *et al.*, 2012; Mustafa and Ozaydjn, 2013).

Other than a structure providing nutritional support for the anangiotic retina (Rodriguez-Peralta, 1975), the *pecten oculi* has been hypothesized to function in intraocular pH and pressure regulation (Brach, 1975), stabilization of the vitreous body (Tucker, 1975), reduction of intraocular glare and maintenance of intraocular pressure (Seaman and Himelfarb, 1963), and in maintenance of the blood ocular barrier for the retina and vitreous body (Barlow and Ostwald, 1972). Color and pulsed Doppler ultrasonography have been used to further investigate the function of *pecten oculi* by measuring blood velocity parameters (BVPs) such as peak systolic velocity (V_{max}), end diastolic velocity (V_{min}), pulsatility index (PI), resistive index (RI) and time-averaged maximum frequency (TAMax) (Ferreira *et al.*, 2015). These parameters may assist to evaluate vascular integrity in many organs and tissues (Carvalho and Chammas, 2011; Ferreira *et al.*, 2015). Moreover, it is known that RI and PI values are directly related to the level of metabolism within a particular tissue or organ. When diastolic velocity increases relative to systolic velocity, RI and PI values decrease (Carvalho and Chammas, 2011). High diastolic velocity suggests

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a high metabolic rate due to a demand for continuous blood flow (Carvalho and Chammas, 2011). Consequently, knowledge of BVPs provides information that is suggestive of the metabolic activity of the *pecten oculi* and may offer insight to its function within the eye (Greenfield *et al.*, 1995; Gellat-Nicholson *et al.*, 1999; Carvalho and Chammas, 2011; Ferreira *et al.*, 2015). To the authors' knowledge, only one study applying this concept using Doppler imaging ultrasonography of the *pecten oculi* was previously performed on American pekin ducks (*Anas platyrhynchos domestica*) (Ferreira *et al.*, 2015).

The objective of this investigation was to evaluate the BVPs in the *pectinis oculi* artery of harpy eagles in order to make conjectures about the metabolic activity of the *pecten oculi* and its function. A second objective was to verify these earlier findings and help establish repeatability and validity of using Doppler imaging ultrasonography as a reliable method to assess metabolic activity of ocular tissues.

Materials and Methods

This study was approved by the Federal University of Paraná's Animal Welfare Committee and was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals

Twenty harpy eagles, born and raised in captivity, consisting of 13 males and seven females (confirmed by cytogenetics) were evaluated. All of the birds were born and belonged to the ITAIPU BINACIONAL's Biological Sanctuary, located on the border between Brazil and Paraguay, in the State of Paraná, Brazil. The birds had a mean weight of 5.85 ± 1.2 kg, and a mean age of 41.94 months (ranging from 12 to 88 months). Only individuals with no evidence of ocular abnormalities following slit lamp biomicroscope (Hawk Eye; Dioptrix, L'Union, France) and indirect ophthalmoscope (EyeTech; São Paulo, Brazil) evaluation were included in the study. All ophthalmic examinations were performed by the same veterinary ophthalmologist (TF).

Ocular Doppler Ultrasonography

All animals in this study are handled and manually restrained regularly as part of their husbandry and management program. This practice conditions them to handling, thus facilitating veterinary examinations and treatments, and reduces the overall stress of the birds. For all procedures described herein, all animals were physically restrained by experienced and well-trained personnel wearing adequate protective equipment (leather gloves and aprons). All birds were hooded just prior to having measurements taken, with claws closed with adhesive tape and beak closed manually by the examiner.

Blood velocity parameters in the *pecten oculi* were evaluated using an ultrasound system (Logiq 5 GE;

Chicago, United States) coupled with a high-frequency 12 MHz ultrasound transducer in both eyes. After instillation of one drop of a topical analgesic solution (proximetacaine 0.5% - Anestalcon - Alcon do Brasil, São Paulo, Brazil), ultrasonic gel (Carbogel, São Paulo, Brazil) was applied over the corneal surface. The ultrasound transducer was perpendicularly positioned in a horizontal plane, from 4 to 10 o'clock. The long axis of the transducer was placed on top of the corneal surface (transcorneal technique), with the marker pointing nasally and inclined 45°. First the base of the pecten was observed by B-mode ultrasound, near the optic nerve. Doppler settings used were: frequency (5.0 MHz), gain (24.5 dB), pulse repetition frequency (1.4 KHz) and wall filter (89 Hz). The *pectinis oculi* artery was located on the more nasal portion using the power Doppler mode to inspect the blood flow. The formulas to determine the resistive index and pulsatility index for the *pectinis oculi* artery were $RI = (V_{max} - V_{min}) / V_{max}$ and $PI = (V_{max} - V_{min}) / T_{MAX}$, respectively (Greenfield *et al.*, 1995; Gellat-Nicholson *et al.*, 1999; Carvalho and Chammas, 2011; Ferreira *et al.*, 2015), where RI= resistance index; V_{max} = the maximum, peak systolic velocity of the *pectinis oculi* artery; V_{min} = the minimum, end diastolic velocity of the *pectinis oculi* artery; PI= pulsatility index; and; T_{MAX} = is the time-averaged mean of the maximum velocity. RI values can vary from 0 to 1 (Nelson and Pretorius, 1988; Martinoli *et al.*, 1998; Gellat-Nicholson *et al.*, 1999; Brooks *et al.*, 2007).

Statistical Analysis

Descriptive statistics of blood flow parameters is presented. The normality of the distribution of residuals was tested graphically and confirmed by a p value > 0.05 on the Shapiro-Wilk test. T -tests were performed to compare values obtained on right and left eyes and on males and females. Differences were considered significant when $P < 0.05$. Statistical analysis was performed using StatView (StatView, Mountain View, CA).

Results

Doppler ultrasonography was successfully performed in 39/40 eyes (one eye had a severe corneal lesion at the time of the Doppler investigation and was excluded from the study). During ultrasonography the pecten was easily located in the posterior segment of each eye as a hyperechoic structure emerging directly anteriorly from the optic nerve. The *pectinis oculi* artery was identified directly underneath the most nasal portion of the *pecten oculi* and Doppler blood velocity parameters were determined. Ocular Doppler ultrasonography examination allowed images to be obtained of the *pectinis oculi* artery in spectral Doppler mode (Fig. 1A). The artery was located directly underneath the nasal portion of the *pecten* and was clearly identified for all BVP measurements (Fig. 1B).

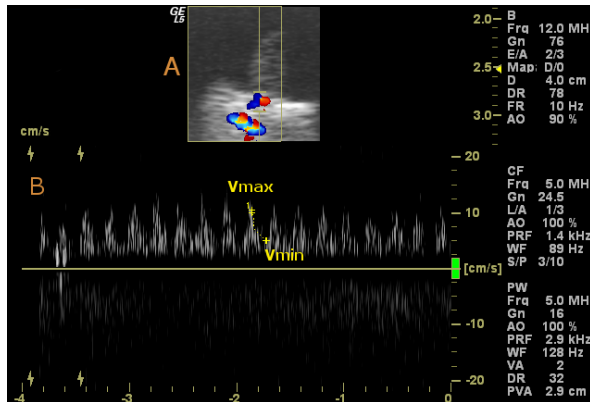


Fig. 1. Spectral (Pulsed) Doppler images. (A): Detection of *pectinis oculi* artery in the most nasal portion of *pecten oculi*; (B): Doppler waves indicated as Vmax (peak systolic velocity) and Vmin (end diastolic velocity).

All blood velocity parameters are described in Table 1. No significant differences between right and left eyes were found, and no significant differences were found between sex and age of the harpy eagles ($P>0.05$).

Table 1. *Pectinis oculi* artery blood velocity parameters of twenty harpy eagles (*Harpia harpyja*).

Both eyes (n=39)	Mean	Variance	Std. Dev.	Std. Err
Vmax	15.34	30.457	5.519	0.884
Vmin	2.912	17.87	4.227	0.677
RI	0.44	0.011	0.103	0.017
PI	0.618	0.042	0.204	0.033
TAmx	11.174	23.129	4.809	0.77

Vmax - peak systolic velocity; Vmin - end diastolic velocity; RI - pectinate artery resistive index; PI - pulsatility index; TAmx - time-averaged maximum frequency.

Discussion

Investigations of BVPs have been made in many organs and tissues in different species such as cat (Carvalho, 2009; Reis *et al.*, 2014), chicken (Barua *et al.*, 2007), dog (Lamb *et al.*, 1999; Lee *et al.* 2014; Souza *et al.*, 2014), rabbit (Abdallah *et al.*, 2010), crab-eating foxes (Silva *et al.*, 2014) and more recently, in ducks (Ferreira *et al.*, 2015). Assumptions about a tissue's metabolism can be made from its blood supply's Vmax, Vmin, RI and PI parameters (Carvalho and Chammas, 2011). Higher metabolism is signified by lower RI and PI values, whereas low or slower metabolism is signified by higher RI and PI values (Carvalho and Chammas, 2011).

For the purpose of comparison, the anterior ciliary artery and short posterior ciliary artery in dogs have been shown to have RI values of 0.53 and 0.44 respectively, the first supplies blood to the ciliary body whereas the second to the choroid and retina that have

higher metabolism than ciliary body (Gellat-Nicholson *et al.*, 1999).

RI values of the long posterior ciliary artery (LPCA) have been demonstrated in the rabbit and the dog as a means to evaluate the importance of the LPCA as a vascular supply to the retina, which is a very highly metabolic tissue (Gellat-Nicholson *et al.*, 1999; Abdallah *et al.*, 2010; Ferreira *et al.*, 2015). The rabbit has been shown to have a very low RI (0.09 ± 0.05) compared to the dog (0.51 ± 0.006), suggesting that the LPCA is of greater importance as a vascular supply to the non-central region of the merangiotic rabbit retina than in the holangiotic retina of dogs (Tokoro, 1972; Bill, 1985; Gellat-Nicholson *et al.*, 1999; Samuelson, 2007; Abdallah *et al.*, 2010; Yang *et al.*, 2011; Ferreira *et al.*, 2015).

A previous study of the RI and PI values of the *pectinis oculi* artery in American pekin ducks showed similar RI and PI values of the short posterior ciliary artery that supplies the ciliary body in the dog (Gellat-Nicholson *et al.*, 1999; Abdallah *et al.*, 2010; Ferreira *et al.*, 2015). The RI and PI values of the *pectinis oculi* artery in harpy eagles found in the present study were also similar. Considering these findings, perhaps there are similarities between the function of the *pecten oculi* and ciliary body, as suggested by the previous theory that the *pecten oculi* could be used for maintenance of IOP through fluid production/excretion into the eye (Seaman and Himelfarb, 1963).

However, another investigation contradicts this theory, as they found several structures that would suggest otherwise: choroidal lacunas within the endothelium, absence of well-delimited basal lamina muscular tunica, innervation and acellular material filling their lumens, and the same characteristic of lymphatic vessels (De Stefano and Mugnaini, 1997). Furthermore, the choroidal lacunae become smaller and less numerous near the optic nerve, *pecten oculi*, and iridotrabecular angle, and therefore are not a part of a Schlemm's canal (De Stefano and Mugnaini, 1997). Several other observations made on the *pecten oculi* also give support to the hypothesis of a secretory function, including 1) extrusion of dye from the pecten accompanying each heart beat during fluorescein angiography (Bellhorn and Bellhorn, 1975), 2) the pecten's high content of carbonic anhydrase and alkaline phosphatase (Bawa and YashRoy, 1972; Amemiya, 1982), 3) evidence that the pecten acts as an agitator to propel perfusate towards the central retina (Pettigrew *et al.*, 1990), and 4) evidence of the pecten having a crucial role in maintaining retinal health (appearance of retinal damage after *pectin oculi* ablation (Wingstrand and Munk, 1965).

In this study, we showed that Doppler imaging is capable of successfully enabling characterization of the

main hemodynamic features of the *pectinis oculi* artery in harpy eagles.

Based on the evidence provided here, i.e. high BVP results, as similarly reported in American pekin ducks, we provide indirect evidence for a potential secretory function of the *pecten oculi*. Future studies should also specifically evaluate the *pecten oculi* using other methods of investigation and in other species of birds.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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