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Effects of pupil dilation with topical 0.5% tropicamide on retinal vascular parameters assessed by VAMPIRE® software in healthy cats.

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Highlights

- Retinal circulation can be considered as a window into the systemic vasculature and can be monitored directly and repeatedly over time by computerized image analysis of fundus photography.
- For a thorough investigation of retinal vasculature, tropicamide is commonly used as a topical mydriatic agent in veterinary ophthalmology.
- We investigated the effects of mydriasis obtained with topical tropicamide on feline retinal vascular parameters evaluated with VAMPIRE® imaging software.
- Our results showed that local tropicamide seems to be associated with a small retinal arteriolar vasoconstriction as assessed by VAMPIRE® in cats.
- Changes in arteriolar caliber are minimal, and should not affect the interpretation of the results when using VAMPIRE® imaging software.

Abstract

Our study investigates the effects of mydriasis obtained with topical 0.5% tropicamide on retinal vascular parameters evaluated in cats using the retinal imaging software: Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE®). Forty client-owned healthy adult cats were included in the study. Topical 0.5% tropicamide was applied to dilate only the right pupil. The left eye was used as a control. Before dilation (T0), infrared pupillometry of both pupils was performed and ***fundus oculi*** images were taken from both eyes. Right eye fundus images were then captured 30 minutes after topical application of tropicamide (T30), when mydriasis was achieved. The retinal vessel widths (3 arteries and 3 veins) were measured with VAMPIRE® in four standard measurement areas (SMA) identified with the letters A, B, C, D. **Average value of the 3 vessel widths was used**. After normality assessment, the t-test was used to analyse the mean difference in vascular parameters of the left and right eyes at T0 and T30, **with *p* set < 0.05**. The two eyes showed no statistical differences in pupil and vascular parameter measurements at T0. At T30, only one artery measurement of the right eye (SMA A-peripapillary area) showed a small but statistically significant mean vasoconstriction of approximately 4%. The results indicate that local application of 0.5% tropicamide seems to be associated with a small retinal arteriolar vasoconstriction as assessed by VAMPIRE® in cats. However, this change is minimal, and should not affect the interpretation of the results when VAMPIRE® is used.

Key Words: cat, ***fundus oculi***, tropicamide, retinal vessels, retinal imaging analysis, VAMPIRE®

1 Introduction

Retinal circulation can be considered as a window into the systemic vasculature (Ikram et al., 2004; Ikram et al., 2006; Wang et al., 2007), which is essential for understanding the effects of systemic diseases such as hypertension or diabetes both in humans and animals (Carter et al., 2014; Cheung et al., 2012; Cirla et al., 2019; Cirla et al., 2021; Crispin and Mould, 2001; Harring, 2021; Ikram et al., 2004; Ikram et al., 2006; Maggio et al., 2000; McGeechan et al., 2009; McKay et al., 2018; Wang et al., 2007; Wong et al., 2001). In vivo vasculature in the retina can be directly visualized and monitored repeatedly over time. For a better evaluation of the ***fundus oculi*** which includes a complete observation of the retinal vasculature, pharmacological pupil dilation is advocated.

Tropicamide is commonly used as a topical mydriatic agent in both human (Frost et al., 2019; Harzany et al., 2013; Vuori et al., 1994; Wang et al., 2018) and veterinary ophthalmology (Harring 2021; Rubin and Wolfes, 1962; Stadtbaumer et al., 2002; Stadtbaumer at al., 2006). It is a parasympatholytic agent that blocks the muscarinic acetylcholine receptors, resulting in the inhibition of the responses of the sphincter muscle of the iris and the ciliary muscle to cholinergic stimulation. The effect is reversible mydriasis and paralysis of the ciliary muscle of the eye. Maximum pupil dilation induced by tropicamide appears within 15–30 minutes, and lasts for approximately 3–8 hours in humans (Frost et al., 2019; Vuori et al., 1994; Wang et al., 2018). In cats, a single application of 0.5% tropicamide determines mydriasis within 15 minutes, reaching the maximum effect within 1-2 hours. **Maximal mydriasis due to tropicamide typically lasts from 2 to 4 hours** (Harring, 2021; **Stadtbaumer et al., 2006**).

By virtue of advances in technology, computerized image analysis of fundus photography has led to the non-invasive evaluation and characterization of blood retinal vessels, also in veterinary medicine (Abtamoff et al., 2010; Cirla et al., 2019; Enache et al., 2021; Hubbard et al., 1999; Trucco et al., 2013). To facilitate fundus image capture for computerized analysis, achieving pupil dilation through the topical use of a parasympatholytic drug such as tropicamide is useful. Pupil

dilatation also enables more peripheral fundus areas to be observed and to acquire images that would otherwise remain unevaluated. However, studies in humans have demonstrated that topical tropicamide reduces retinal vessel diameters (Wang et al., 2018) or retinal capillary blood flow (Harzany et al., 2013). Consequently, the results of these latter studies suggest that pharmacologically-induced mydriasis should be avoided when performing computerized fundus image analysis. In contrast, Frost et al. (2019) failed to find evidence of the vasoconstriction effect of tropicamide on retinal vasculature, and a blood flow study on macula capillaries showed no changes due to tropicamide (Robinson et al., 1985).

In veterinary medicine tropicamide is the drug of choice for diagnostic mydriasis, because of its rapid onset, completeness of pupil dilation, and short-acting properties (Harring, 2021; Rubin and Wolfes, 1962; Stadtbaumer et al., 2002; Stadtbaumer et al., 2006). **Other mydriatic topical agents did not show the same characteristics in cats (Stadtbaumer et al., 2006).** Considering the emerging interest in computerized image analysis of ***fundus oculi***, especially in cats (Cirla et al., 2019; Cirla et al., 2021; Enache et al., 2021), and the difficulty in collecting good quality images with undilated pupils without making the procedure too long, we wanted to verify whether measurements of retinal vessel caliber were the same in the images taken before and in those taken after pharmacological mydriasis obtained with tropicamide in the feline eye.

This study thus investigated whether pupil dilation with topical 0.5% tropicamide changed the retinal vascular parameters in cats evaluated by the retinal imaging software Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE®). Our null hypothesis was that tropicamide induces mydriasis without affecting retinal vessel parameters assessed by VAMPIRE® in healthy cats, and therefore tropicamide could be used whenever images for measuring retinal vessels are needed.

2 Materials and methods

Adult cats presenting for a clinical check-up were enrolled in the study on the basis of the results of a physical and ophthalmological examination. Approval for this study was obtained from the Institutional Animal Care and Use Committee of the University of Pisa (D. lgs.vo 26/2014). Informed consent was obtained from all the owners after a full explanation of the study. All cats included in the study were domestic short-haired with a median weight of 5.3 kg (ranging from 4.2 to 5.9 kg).

Anamnestic and clinical information were analyzed to rule out current or prior systemic diseases. Complete blood count (CBC), complete biochemical and coagulation profile, serum electrophoresis urine test (urinary dipstick, specific gravity on refractometer, osmolarity, urinary protein to urinary creatine ratio, microscopic sediment) were available for each cat enrolled.

No cats had been given any medication known to affect the autonomic, cardiac or vascular system. All cats underwent a physical examination that included measurements of the cardinal signs (body temperature, pulse and respiratory rate), hydration status, thoracic auscultation, and abdominal palpation. Systemic pressure was assessed using high-definition oscillometry (petMAP®, Ramsey Medical Inc, Tampa, FL). The same operator carried out three sequential measurements at one-minute intervals. Blood pressure values (systolic and diastolic) were calculated as the mathematical mean of the three measurements. The measurements taken from agitated or moving cats were eliminated, as were those where the heart rate measured with the oscillometer differed from the heart rate measured manually by more than 50 beats per minute.

Prior to inclusion in the study, all cats were also determined to be ophthalmologically normal by a thorough examination including slit-lamp biomicroscopy (SL-15 portable Slit lamp, Kowa Company, Tokyo, Japan), as well as indirect ophthalmoscopy (Heine Omega 500 Unplugged and Heine 30D lens; Heine Instruments, Herrsching, Germany) performed in a dark room without pharmacological pupil dilatation. All tonometric measurements were performed using a rebound

tonometer (TonoVet, iCare, Vantaa, Finland) **thus avoiding the use of ophthalmic topical anesthetic agents**.

Pupil diameter measurements (O.S.A. Vet, SBM Sistemi, Orbassano, TO, Italy) and disc centered fundus photographic documentation (Clearview, Optibrand LLc, Ft Collins, Columbia, United States) were performed on both eyes (OU) before pharmacological dilation (T0). Only the right eye (OD) was dilated with one drop of 0.5% tropicamide (Visumidriatic 0.5%, Visufarma s.p.a., Rome, Italy), while the left eye (OS) was used as a control. Pupil diameter measurement and retinal imaging of both the dilated OD and the non-dilated OS were performed again 30 minutes after 0.5 % tropicamide application (T30). Any images with defects caused by movement **of the animal** were excluded. All testing was performed during a single examination. Blood pressure values were taken again at T30.

The VAMPIRE® annotation tool adapted for measuring the feline ***fundus oculi*** was then used to evaluate retinal vessel widths (3 arteries and 3 veins). All the measurements were carried out by the same trained operator (AC) using a previously described method (Cirla et al., 2019; Trucco et al., 2019) (Fig. 1).

All clinical data were catalogued using P.O.A.® System Plus software (v. 254).

Descriptive statistics were performed for the variables: sex (male/female), age (years), pupil diameter (mm), and vascular measurements (pixels). After normality assessment, the t-test was used to analyse the mean difference in OS and OD vascular parameters at T0 and T30. The Student's paired *t*-test was used to compare the mean difference in OS and OD vascular parameters at T0 and T30, as well as the mean systemic blood pressure at T0 and T30.

Statistical significance was set at $p < 0.05$ with Bonferroni correction accounting for multiple comparisons.

3 Results

A total of 40 healthy domestic short haired cats were included in the study, composed of 22 spayed females and 18 neutered males. **In each animal included, no alterations were observed in both the anterior and posterior segments of the eye, as well as the IOP values were always within the range considered normal for the feline species (Rusanen et al., 2010)**. The mean age was 5.7 years, with a median age of 5.6 years (range from 4.6 to 7.6 years). No adverse reactions or events were observed due to tropicamide topical administration or study procedures.

At T0 the mean OD pupil diameter was 6.08 mm (standard deviation 0.80, standard error of the median 0.12), while the mean OS pupil diameter was 6.13 mm (standard deviation 0.80, standard error of the median 0.12). Both eyes showed no statistical differences in pupil (p = 0.79) and retinal vascular parameters in each standard measurement area (SMA), as reported in Table 1.

At T30 the pupil diameter of the right eye increased (p <0.001) and was 9.71 mm (standard deviation 0.67, standard error of the median 0.10) with a percentage variation (T0-T30) of 59.6%. The pupil diameter of the left eye was not significantly different (p=0.77) from the T0 values.

Vascular parameters in OD and OS at both T0 and T30 are reported in Table 2. At T30, only one mean artery measurement (SMA A, OD) showed a small but highly significant (p<0.001) vasoconstriction of approximately 4% (exact value 3.82%, 6.28±0.84 *vs* 6.04±0.59) as none of the other vascular parameters were significantly different at this timepoint (Fig. 2). Blood pressure showed no significant changes before (T0) and 30 minutes after (T30) the topical application of tropicamide (p=0.71).

4 Discussion

Retinal vasculature offers the unique opportunity to analyse blood microcirculation non-invasively (Cheung et al., 2012; Ikram et al., 2004; McGeechan et al., 2009; Wang et al., 2007; Wong et al., 2001). It could thus be an important research tool in the detection and follow-up of systemic diseases also in animals such as cats, as recently reported (Cirla et al., 2019; Cirla et al., 2021;

191 Enache et al., 2021). Tropicamide is routinely used for pupil dilation to facilitate clinical
192 examination and imaging of the retina both in humans and animals (Frost et al., 2019; Harzany et
193 al., 2013; Rubin and Wolfes, 1962). However, a better understanding of the effects of this
194 commonly used mydriatic agent on the retinal vasculature is crucial for the accurate interpretation
195 of retinal digital imaging analysis. The use of VAMPIRE® for retinal imaging analysis was recently
196 validated in feline species without inducing pharmacological **pupil** dilation (Cirla et al., 2019). The
197 aim of our study was therefore to investigate whether pupil dilation with tropicamide influences the
198 retinal vascular parameters assessed by VAMPIRE® in cats.

199 As expected, 0.5% tropicamide induced pupil dilation by up to 59.6% of the baseline diameter at
200 T30 in the right eye, however the results of the retinal vascular parameters are not completely clear.
201 In fact, contrary to our hypothesis we found a slightly but statistically significant arterial
202 vasoconstriction at the SMA A, which is the area closest to the optic disc. On the other hand, no
203 reduction in the vessel caliber measurements was evident in the veins, which thus complicates the
204 interpretation of our results.

205 In human nitric oxide (NO) has been found to regulate retinal vascular tone in both arteries and
206 veins (Dorner et al., 2003). Similarly, NO as well as other endothelium-derived factors seem to play
207 a role in retinal arteriolar changes due to, for example, hypercapnia (Sato et al., 2003), hyperoxia
208 (Izumi et al., 2008), and acute severe elevation in systemic blood pressure (Nakabayashi et al.,
209 2012) in cats. In addition, the muscarinic receptor M3 has been identified in murine retinal
210 arterioles, mediating cholinergic vasodilation via the activation of NO synthase. By increasing the
211 NO levels, the arteriole induces vasodilation (Gericke et al., 2011). When the receptor M3 is
212 blocked, this vasodilation is not possible, and a vasoconstriction might follow.

213 There are contrasting results in the human literature regarding the effects of topical tropicamide, a
214 blocker of M3 receptors, on the width of retinal vessels assessed by imaging analysis. Some authors
215 have reported a constriction of the retinal vasculature (Harzany et al., 2013; Wang et al., 2018),

which could be explained by the possible vasoactive activity of the drug. It has been **supposed** that 0.5% topically applied tropicamide in humans reaching the retinal vessels of the posterior pole by diffusion **(Harzany et al., 2013), and the same occurrence could happen in animals. However, at this time, no data are available to support this hypothesis.**

However, a recent study demonstrated that despite an apparent reduction in both the arteriolar and venular measurements due to topical tropicamide, this was actually a consequence of a decrease in the size of the colour fundus photography post-tropicamide. The authors speculated that image magnification might be affected by the drug's cycloplegic rather than mydriatic activity (Frost et al., 2019).

In our study we found a minimal but statistically significant peripapillary arterial vasoconstriction, while the veins appeared to be unchanged after tropicamide application. We excluded the possibility that the arterial vasoconstriction could be due to an increase in systemic blood pressure because at T0 all the enrolled cats showed normal values which remained unchanged at T30. It is also true that retinal arterioles respond rapidly to changes in systemic blood pressure, however, in the literature, only a slight reduction in retinal arteriolar diameter has been found to be associated with an acute severe increase in systemic blood pressure in the cat (Nabayashi et al., 2012).

If, on the other hand we assume that tropicamide is a vasoactive retinal drug, the results can be explained by considering a dose-dependent effect. In fact, we used 0.5% tropicamide, a concentration described in the literature as mydriatic in cats (Stadbaumer et al., 2002; Stadbaumer et al., 2006), however lower than that used in studies already published in human medicine regarding the effect of pupil dilation with tropicamide 1% on retinal vascular caliber (Frost et al., 2019; Wang et al., 2018). It is possible that retinal arterioles are more sensitive to the vasoactive effect of the drug, even at low concentrations, and show vasoconstriction. In fact M3 muscarinic receptors have been evidenced in the retinal arteries (Gericke et al., 2011), but not, to the best of our knowledge, to date in the retinal venules. However, it cannot be excluded that topical tropicamide

241 does not affect retinal vascular caliber in cats, and that the peripapillary arterial vasoconstriction
242 detected in our study may be due to the accuracy in precisely defining vessel edges, especially in
243 the arteries, which are smaller than the veins. It should also be emphasized that VAMPIRE® is a
244 semiautomatic software application, and measurements have to be taken manually in very small
245 anatomical structures. Thus, despite the experience of the operator (AC) in the use of the software
246 (Cirla et al., 2019), there may have been some errors when taking the measurements.

247 In our opinion, correlating our results to the fact that tropicamide may affect the magnification of
248 the fundus photography, as reported by Frost et al. (2019) in humans, is less plausible because the
249 vessel width reduction was only evident in the arteries, while the veins were unchanged.

250 The current study has some limitations. First, we used a semiautomatic software application that is
251 prone to human error in measuring very small anatomical structures such as retinal arterioles.
252 Second, the effect of tropicamide was evaluated only at 30 minutes post administration, even
253 though the maximum effect is expected at 1-2 hours. This was because our study was performed in
254 a clinical setting and the aim was to detect whether pupil dilation with topical 0.5% tropicamide
255 changes retinal vascular parameters in cats evaluated by the retinal imaging software VAMPIRE®.
256 However usually ocular fundus examination is performed after local mydriatic drug application, in
257 other words after 20-30 minutes. Further studies are needed to clarify whether longer waiting times
258 after application may somehow influence the parameters of the retinal vessels.

259

260 **4 Conclusions**

261 In conclusion, the results of our study indicate that a local application of 0.5% tropicamide seems to
262 be associated with a statistically significant variation in the retinal arteriolar parameters as assessed
263 by VAMPIRE® in cats. However, the change in retinal arteriolar diameter after the pharmacological
264 dilation was minimal, and, we thus believe that this minimal change should not affect the

interpretation of the results when VAMPIRE® is used. It should also be underlined that the variation in the retinal arteriolar width assessed by VAMPIRE® was not detectable during the indirect ophthalmoscopic examination. Our study thus does not provide enough evidence to conclude that the topical use of 0.5% tropicamide causes clinically relevant retinal arteriolar narrowing.

Further studies are needed to better understand the effect of topical mydriatics on retinal vascular parameters in animals assessed by imaging analysis, perhaps using an automatic tool in order to improve accuracy.

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CRediT authorship contribution statement

Alessandro Cirla: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Michele Drigo:** Methodology, Formal analysis, Writing – review & editing. **Lucia Ballerini:** Methodology, Investigation, Formal analysis. **Emanuele Trucco:** Methodology, Investigation, Formal analysis. **Giovanni Barsotti:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Tables

Table 1 – *p* values of venular and arteriolar parameters at T0 for each SMA. Statistical significance level was set at $p<0.05$

		OD							
		Vein A	Vein B	Vein C	Vein D	Artery A	Artery B	Artery C	Artery D
OS	Vein A	0.69							
	Vein B		1.00						
	Vein C			0.86					
	Vein D				0.17				
	Artery A					0.19			
	Artery B						0.93		
	Artery C							0.81	
	Artery D								0.62

Table 2 – Venular and arteriolar width at T0 and T30 for each SMA. Values are expressed in pixels and represent the mean (standard deviation). Statistical significance level was set at p<0.05

	OD			OS		
	T0	T30	*p*-value	T0	T30	*p*-value
Vein A	8.79 (0.41)	8.88 (0.28)	0.209	8.80 (0.42)	8.86 (0.41)	0.133
Vein B	8.89 (0.38)	8.90 (0.dorner)	0.860	8.78 (0.45)	8.86 (0.31)	0.262
Vein C	8.99 (0.41)	8.98 (0.28)	0.821	9.01 (0.36)	8.90 (0.23)	0.164
Vein D	8.99 (0.54)	9.04 (0.22)	0.494	9.10 (0.94)	9.02 (0.61)	0.557
Artery A	6.28 (0.83)	6.04 (0.59)	**0.001**	6.11 (0.82)	6.04 (0.61)	0.883
Artery B	6.36 (0.77)	6.34 (0.74)	0.189	6.29 (0.72)	6.31 (0.75)	0.986
Artery C	6.35 (0.68)	6.36 (0.67)	0.860	6.52 (0.58)	6.41 (0.60)	0.859
Artery D	6.12 (0.64)	6.16 (0.63)	0.224	6.10 (0.61)	6.12 (0.60)	0.329

Figures (colour needed for both Fig.1 and Fig.2)

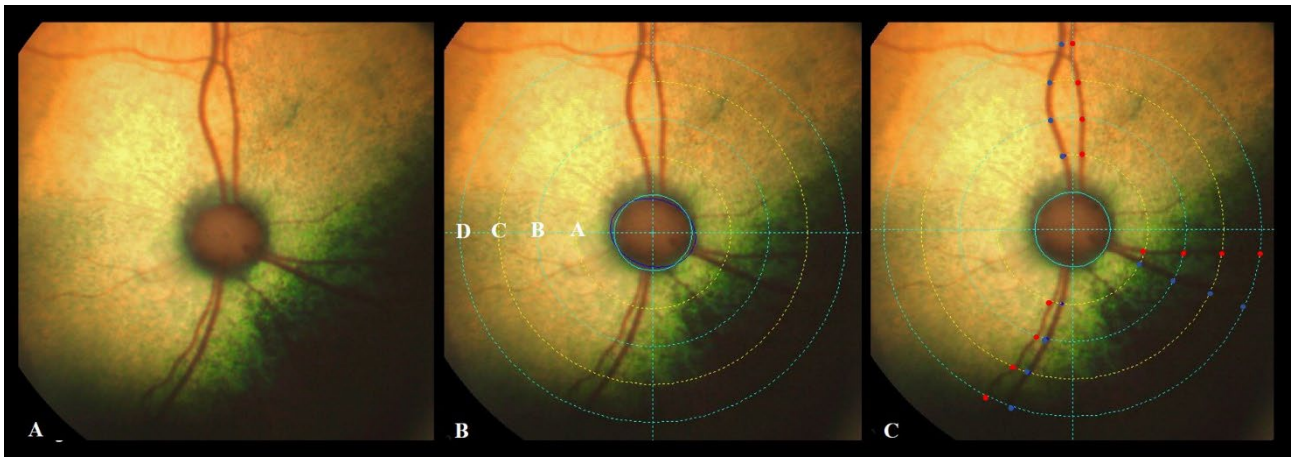
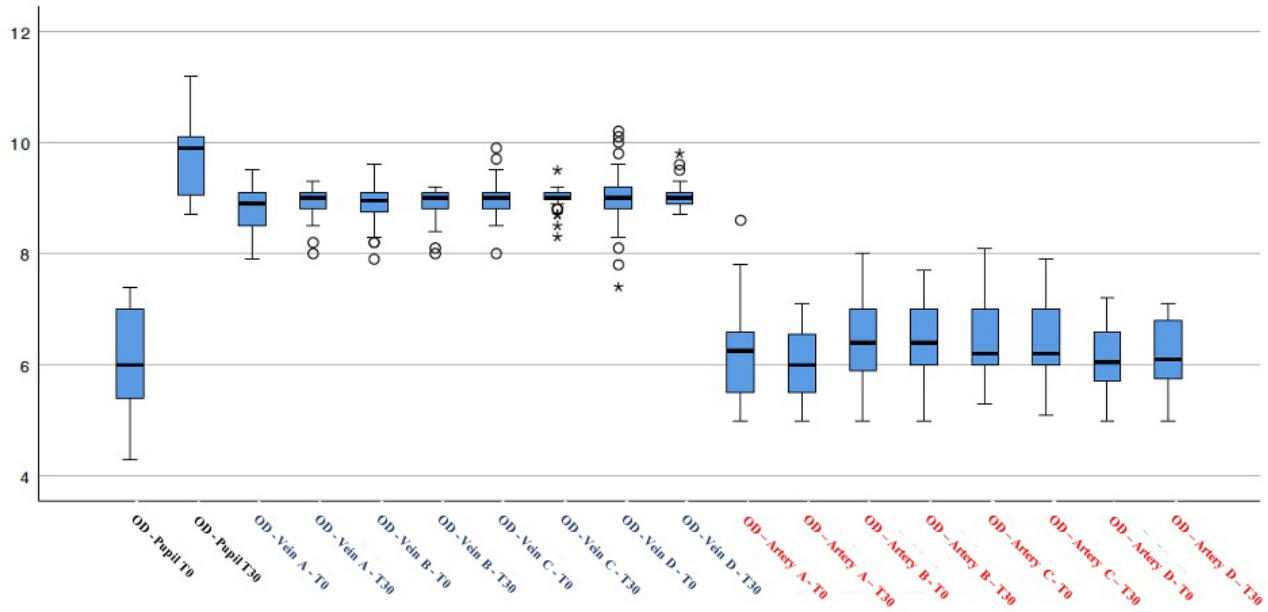


Fig. 1. Image analysis method using VAMPIRE®. A: normal feline optic disc centered fundus image as captured with the fundus camera. B: VAMPIRE® automatically defined the four standard measurement areas (SMA) identified with the letters A, B, C and D. Guidelines (GL) for measuring the vessels (yellow lines) were automatically outlined within each of these areas. C: manual cataloguing the vessel as arterial and venous for the subsequent analysis of their widths. Selecting measuring points of the vessels for each SMA, localized at the intersection between the GL and the vessel itself (arteries are indicated by red dots, veins by light blue dots).



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446 **Fig. 2. Tukey boxplots of the paired comparison of measurements at T0 and T30 for both pupil and retinal**

447 **vessel parameters of the right eye (OD). In blue venular and in red arteriolar parameters for each SMA.**