Effect of nicotinamide on retinal function in glaucomatous dogs with *ADAMTS10*-open-angle glaucoma (*ADAMTS10*-OAG)

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[4] Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

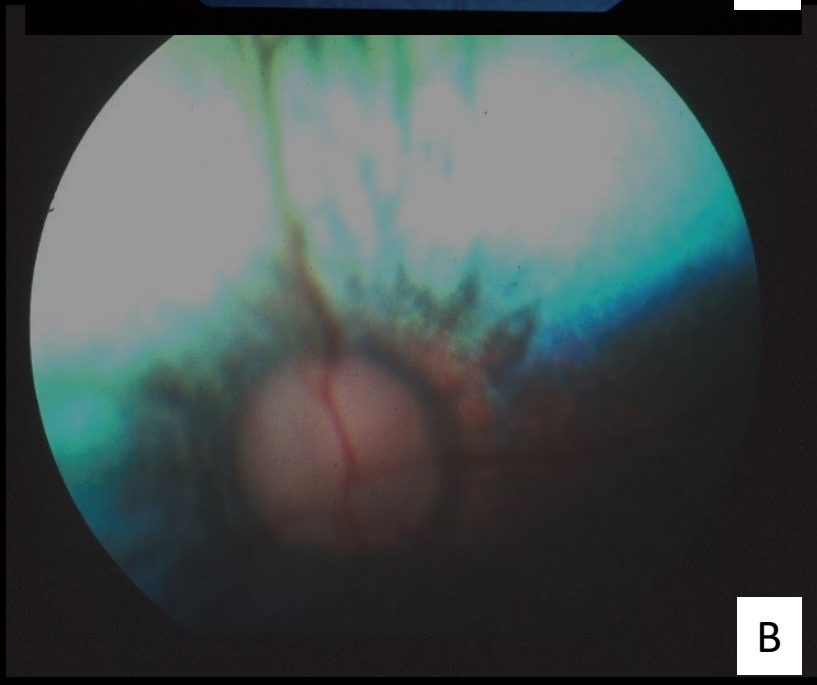
https://my.embarkvet.com/dog/catcher3/gallery#ui%2Frko6d9quhly0v43gushy

Glaucoma

- Effects *many,* yet no cure for *any*
- Leading cause for irreversible blindness worldwide
  - Dogs & humans
    - Estimated prevalence: 1-2%

- Glaucoma = retinal degeneration
  - Retinal ganglion cells (coma or cell death)
  - Optic nerve head (atrophy)

- Risk Factors
  - Increased intraocular pressure
  - Genetics



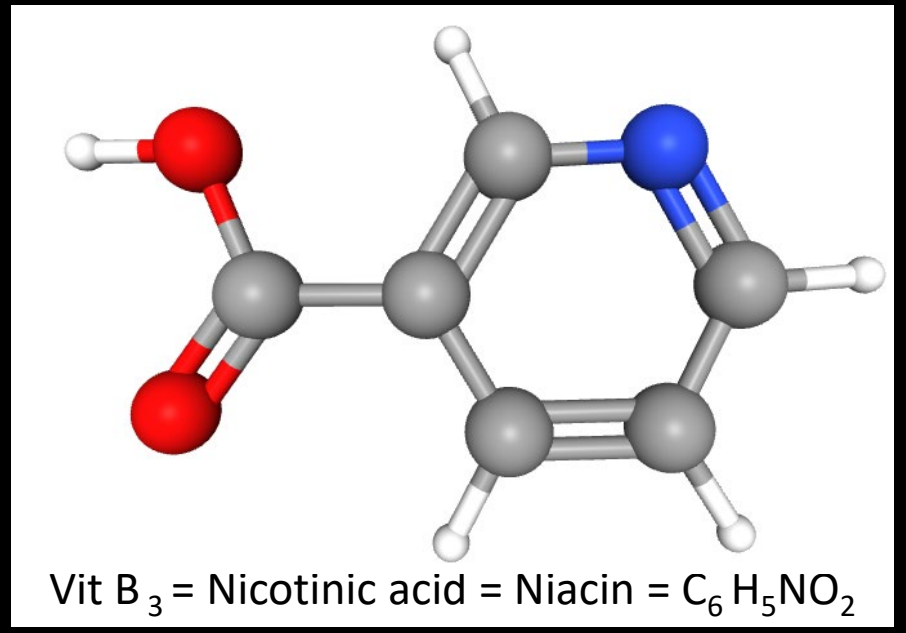
Veterinary Ophthalmology (2004) 7, 2, 97-111.
Gelatt, Veterinary Ophthalmology. (2021) p. 1173
Fry et al.(2018). *Progress in Retinal and Eye Research.***65**, 77-92

Nicotinamide

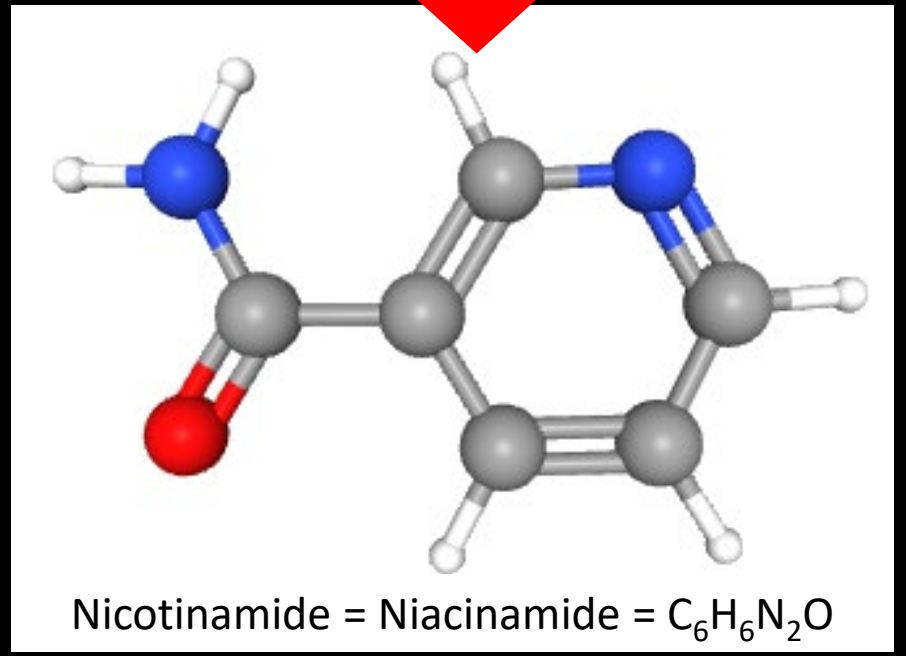
- Dietary Nicotinamide (NAM) supplementation → NAD (Nicotinamide Adenine Dinucleotide)
  - Redox metabolism
- Increased Serum NAD^+ → presumed neuroprotectant for retinal ganglion cells

Previous Studies

- DBA/2J mouse model
- Human



Vit B_3 = Nicotinic acid = Niacin = $C_6H_5NO_2$



Nicotinamide = Niacinamide = $C_6H_6N_2O$

www.pubchem.ncbi.nlm.nih.gov

Tribble et. al.(2021). *Redox Biol.* Online

De Moraes et. al.(2022).*JAMA Ophthalmol.***140**, 11-18.

Hui et. al.(2020). *Clin Experiment Ophthalmol.***48**, 903-914.

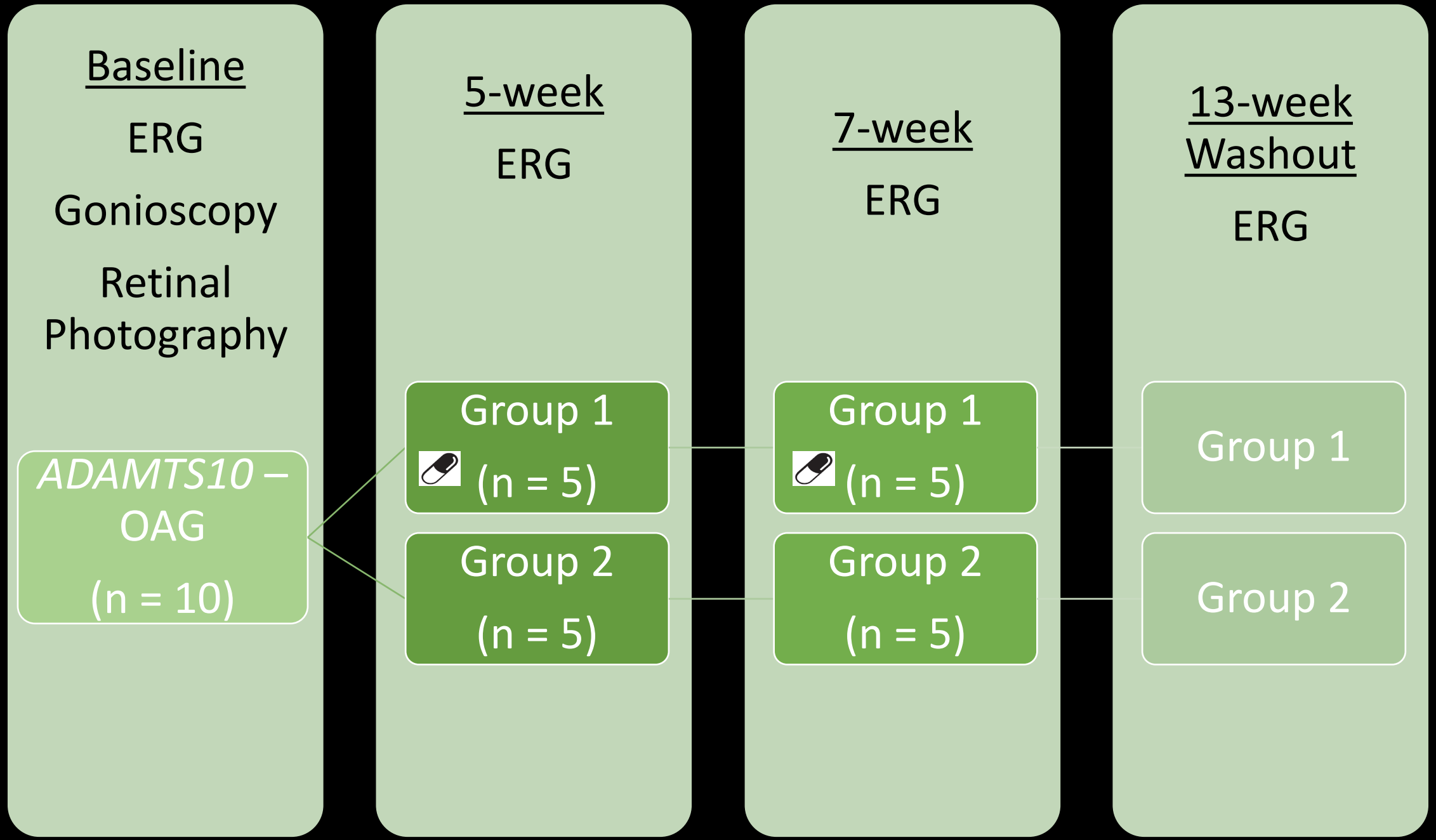
Purpose

This masked, randomized study aimed to demonstrate the effect of 7-week NAM supplementation on inner retinal function using electroretinography (ERG) in *ADAMTS10*-OAG dogs.

Materials & Methods

- IACUC
- 10 adult dogs
- Purpose-bred
- *ADAMTS10* – Primary Open Angle mutant dogs
  - Age, weight, sex

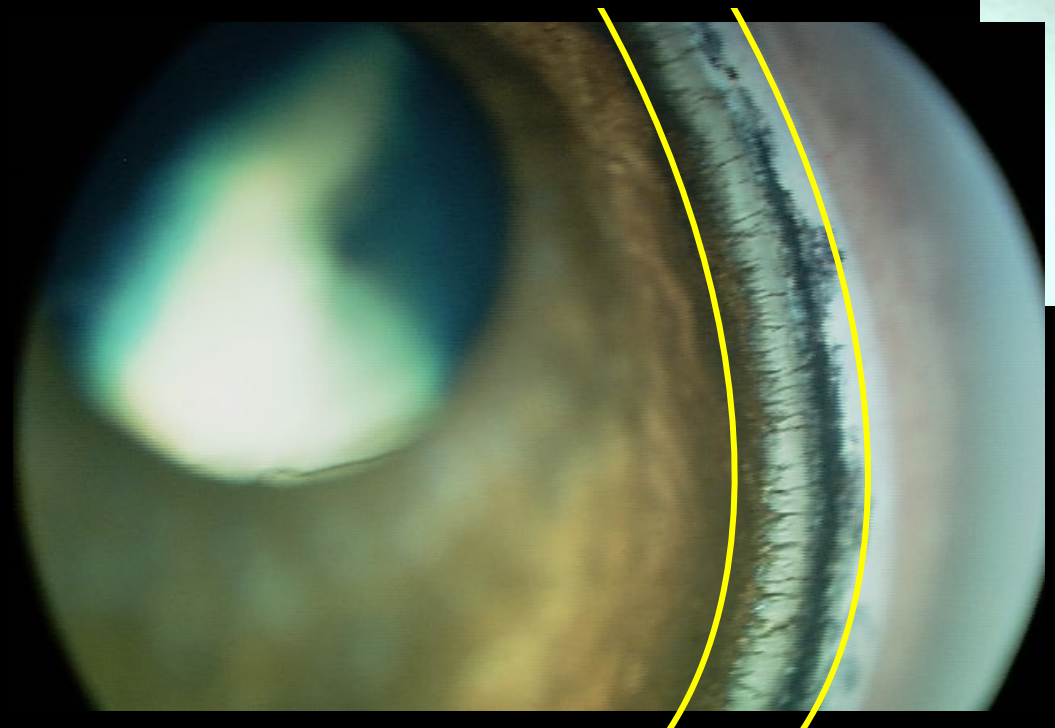
https://www.k9rl.com/dog-breeds/hounds/beagle/

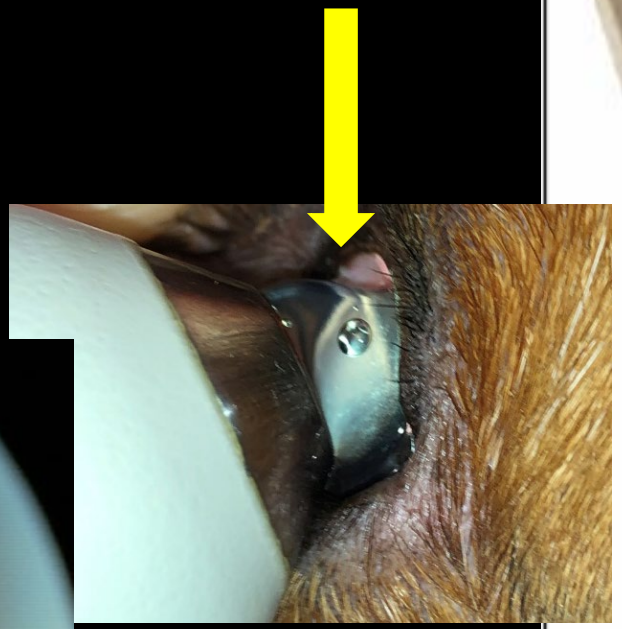

Baseline
ERG
Gonioscopy
Retinal Photography
ADAMTS10 – OAG
(n = 10)
5-week
ERG
Group 1
(n = 5)
Group 2
(n = 5)
7-week
ERG
Group 1
(n = 5)
Group 2
(n = 5)
13-week Washout
ERG
Group 1
Group 2

Materials & Methods

Open-Angle Confirmation

- Gonioscopy
  - RetCam II





Materials & Methods

Normal Diet Maintained

- Teklad 2027
- Purina Pro Plan, Classic Adult
  - Chicken & Rice Entrée

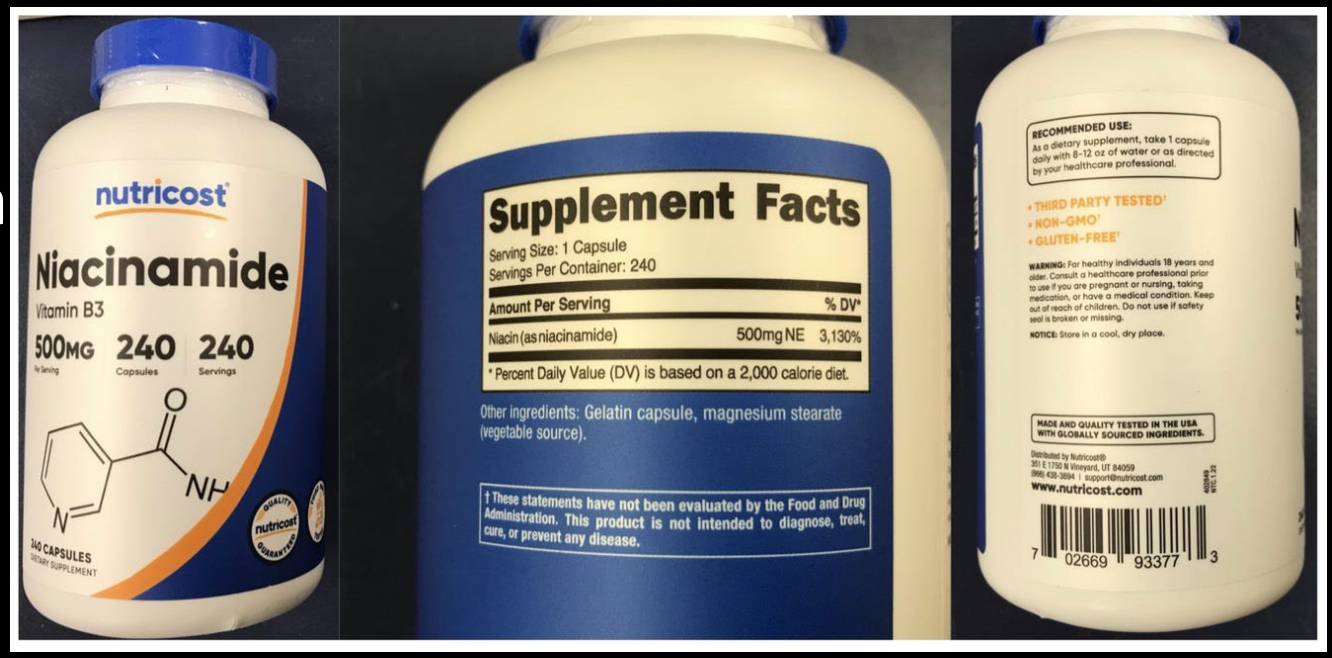
Materials & Methods

NAM Supplementation

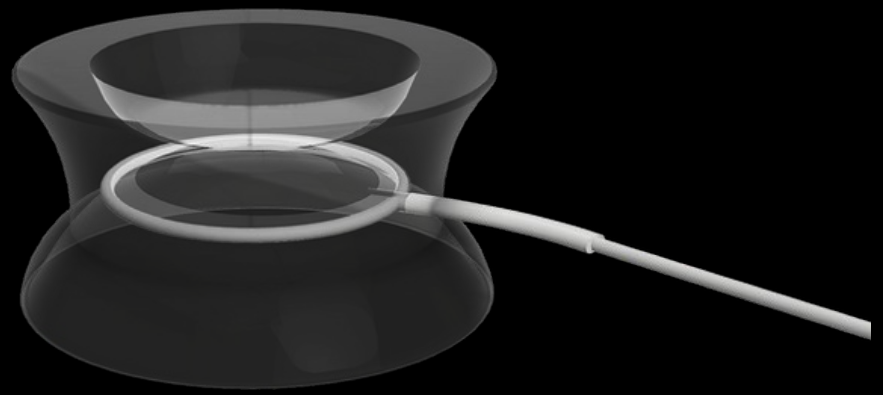
- 1500 mg NAM per day
  - 500 mg NAM (1 capsule) in a meatball
    - 3 times a day
      - 7:00 am, 3:00 pm, 11:00 pm

Sham Supplementation

- 1 ‘empty’ meatball
  - 3 times a day
    - 7:00 am, 3:00 pm, and 11:00 pm



Materials & Methods



Retina Function

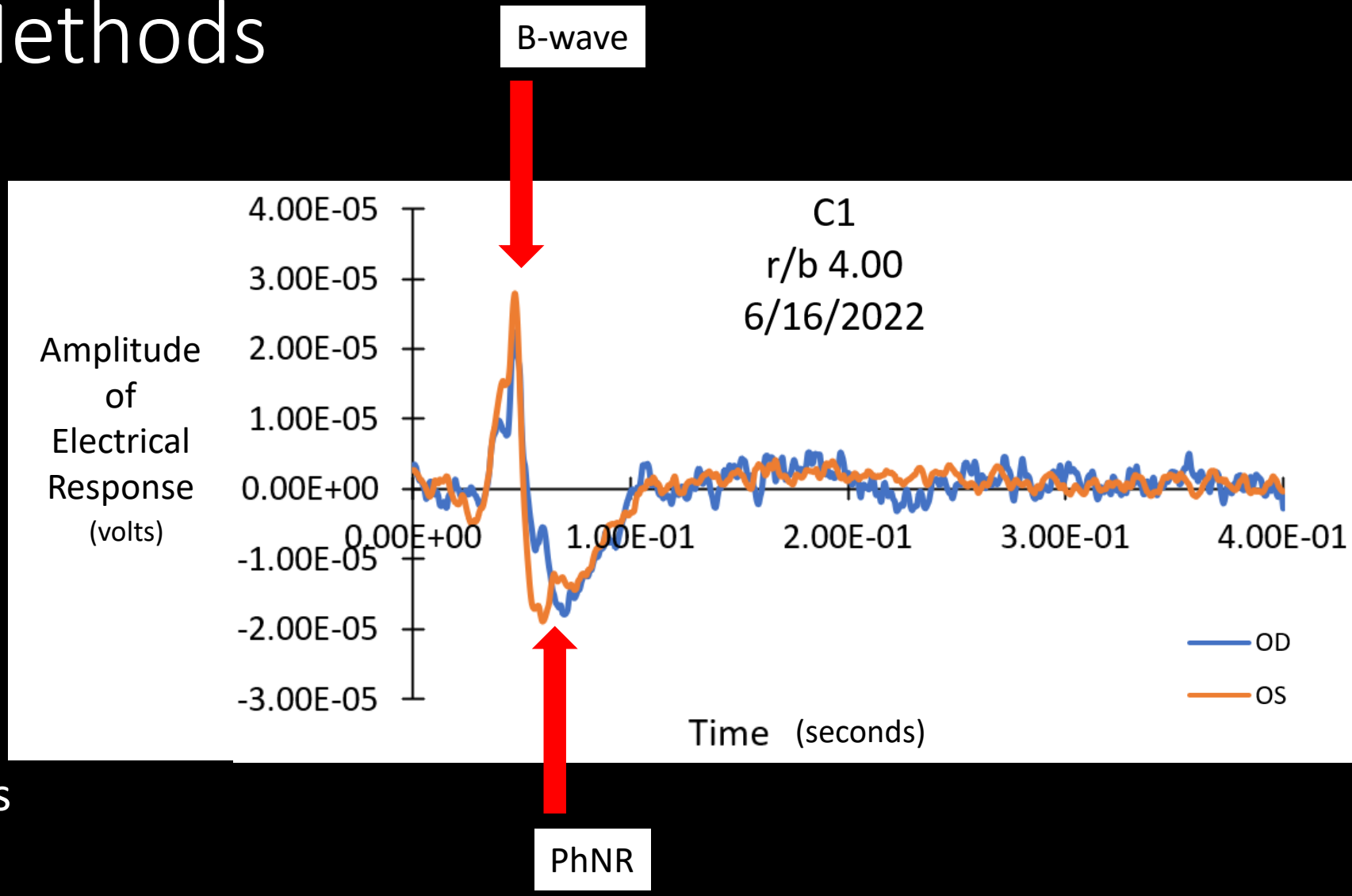
- Electroretinography (ERG)
  - Electrodes: Positive (RM), Negative, and Ground
  - ‘Red on Blue’ flash
    - Blue light adapted (5 minutes)
    - Red flash
      - 0.07, 0.90, 1.74, 2.57, 3.40, 4.00 cd.s/m^2
- ERG Variability
  - Pupil size
    - increased pupil = increased response
  - Intraocular pressure
    - Increased IOP = decreased response

Materials & Methods

Retina Function

- ERG (Right & Left)
  - Time (seconds)
  - Amplitude (volts)

- B-wave:
  - cone bipolar cells
- PhNR:
  - retinal ganglion cells



Pasmanter et al. (2020) *Vet Ophthol.* **23.3**, 418-435.

Materials & Methods

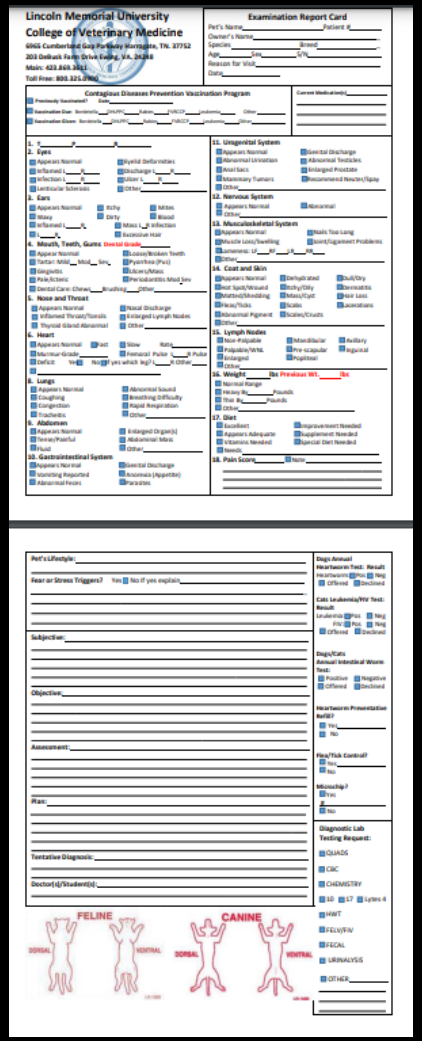
Ophthalmic Monitoring

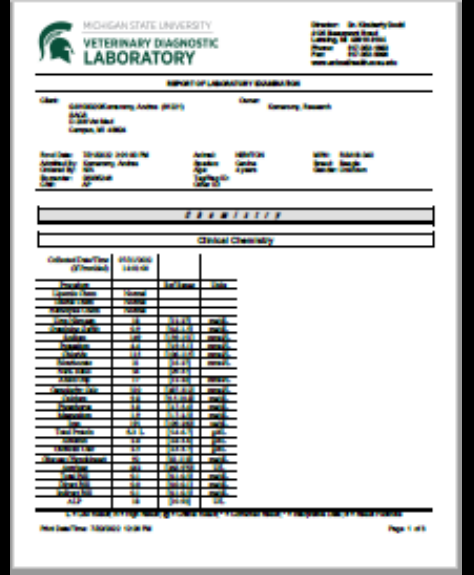
- Ophthalmic Exams
  - Indirect fundoscopy
  - Slit lamp biomicroscopy
- Intraocular Pressure
  - Rebound tonometry
- Retinal Photography

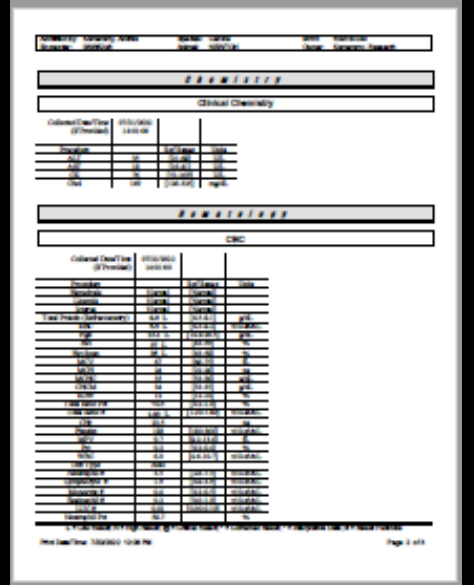
Materials & Methods

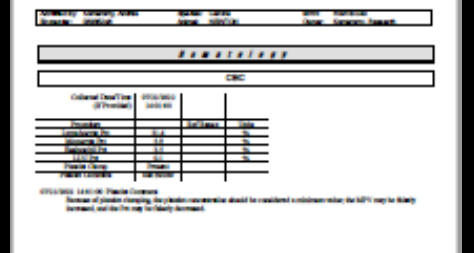
Systemic Monitoring

- Bloodwork (week 5)
  - CBC
  - Chemistry
  - Electrolytes
  - Lutein
- Physical Exams
  - General health
  - Weight









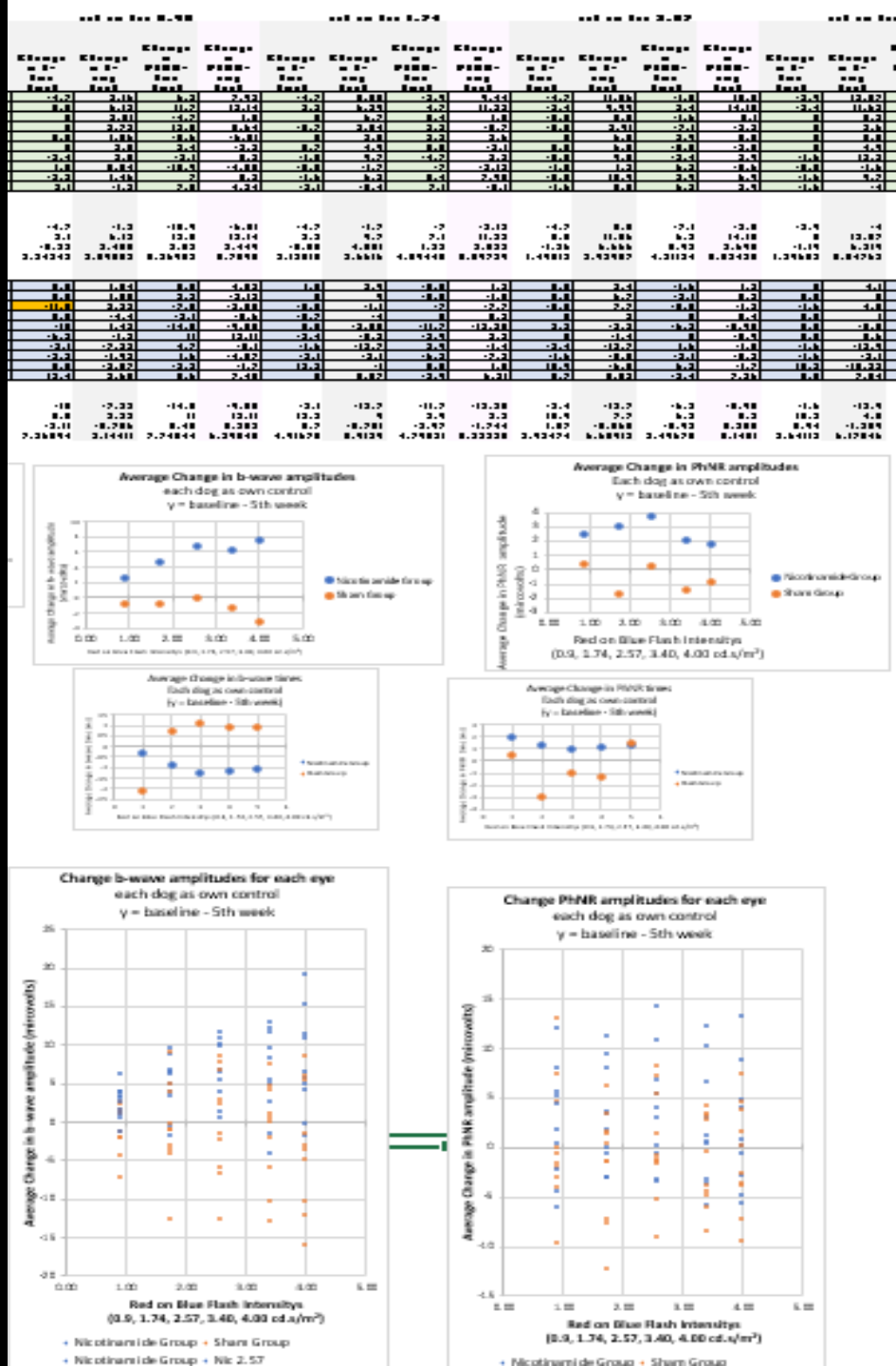
Materials & Methods

Statistical Analysis

- Repeated measures design
- Linear mixed model while controlling for baseline

ERG Comparison

- Baseline (Week 0)
- 5th Week of Supplementation
- 7th Week of Supplementation
- Washout (Week 13)



Results

- Metabolomic results from the aqueous humor will be added to the final publication

Baseline Population Results

Females and males were distributed evenly between groups.

Variable	Control Group (N = 10)[1]	NAM Group (N = 10)[1]	p-value[2]
Sex			>0.99
F	6 / 10 (60%)	6 / 10 (60%)	
M	4 / 10 (40%)	4 / 10 (40%)	

[1] n / N (%); Mean (SD)

[2] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test; Wilcoxon rank sum exact test

Baseline Population Results

Variable	Control Group (N = 10)[1]	NAM Group (N = 10)[1]	p-value[2]
Age (mnth)	50.85 (1.86)	56.39 (12.31)	0.79
Eye			>0.99
Right	5 / 10 (50%)	5 / 10 (50%)	
Left	5 / 10 (50%)	5 / 10 (50%)	

[1] n / N (%); Mean (SD)
[2] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test; Wilcoxon rank sum exact test

Baseline Population Results

Variable	Control Group (N = 10)[1]	NAM Group (N = 10)[1]	p-value[2]
Pupil [mm]	9.00 (0.00)	9.80 (1.14)	0.068
Post-Dilation IOP [mmHg]	35.70 (5.74)	37.00 (6.98)	0.52

[1] n / N (%); Mean (SD)
[2] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test; Wilcoxon rank sum exact test

Baseline Population Results

Variable	Control Group (N = 10)[1]	NAM Group (N = 10)[1]	p-value[2]
b-time [ms]	32.72 (2.85)	31.06 (0.54)	0.007
b-amp [uv]	24.59 (9.42)	23.92 (5.84)	0.91
PhNR-time [ms]	47.67 (3.12)	44.29 (2.25)	0.018
PhNR-amp [uv]	18.90 (4.54)	19.68 (4.73)	0.60
Ratio of PhNR-amp [uv] / b-amp [uv]	0.82 (0.15)	0.83 (0.12)	0.91

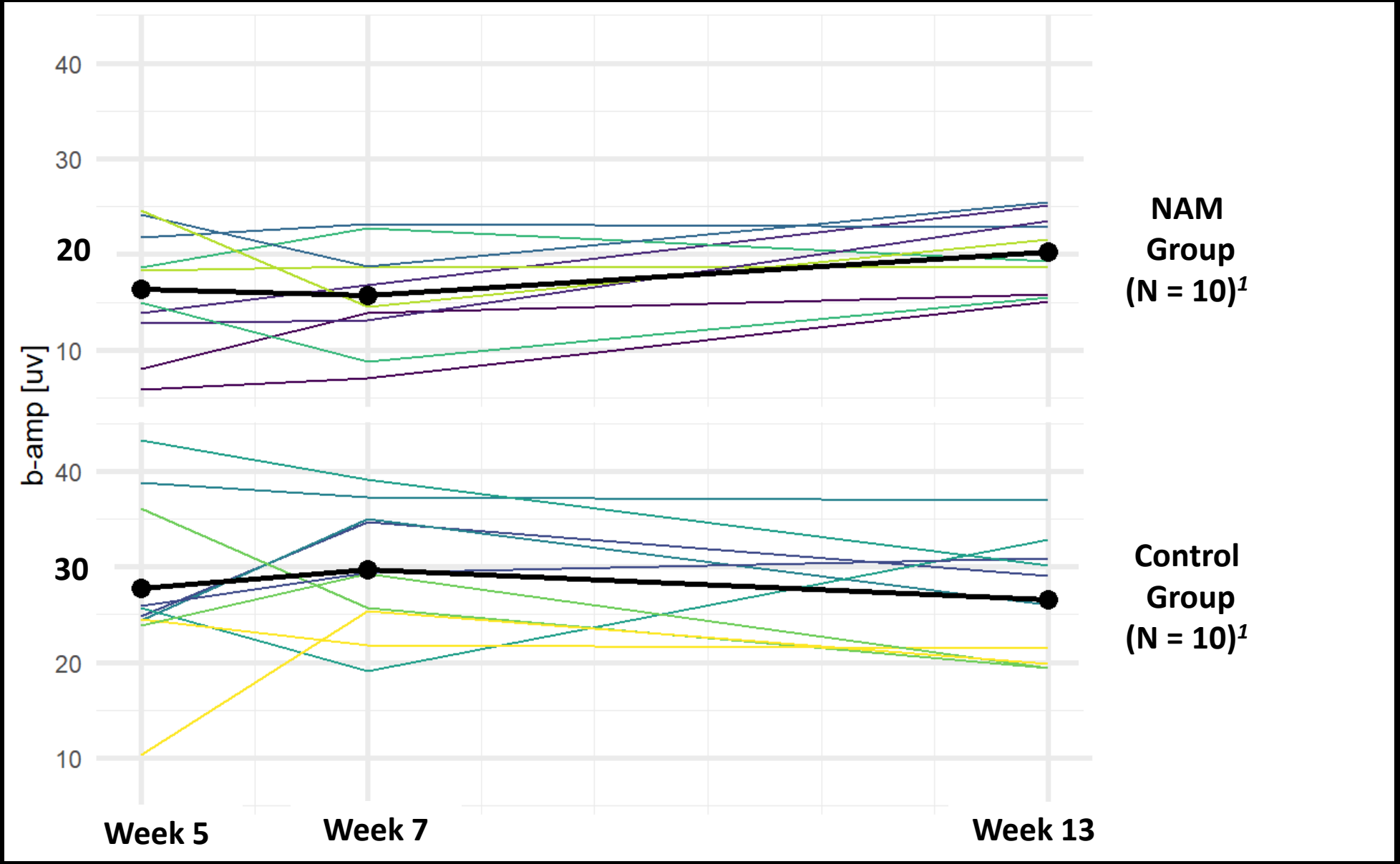
[1] n / N (%); Mean (SD)
[2] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test; Wilcoxon rank sum exact test

Table 4. After treatment Measurement by Treatment and Week(combined two eyes)

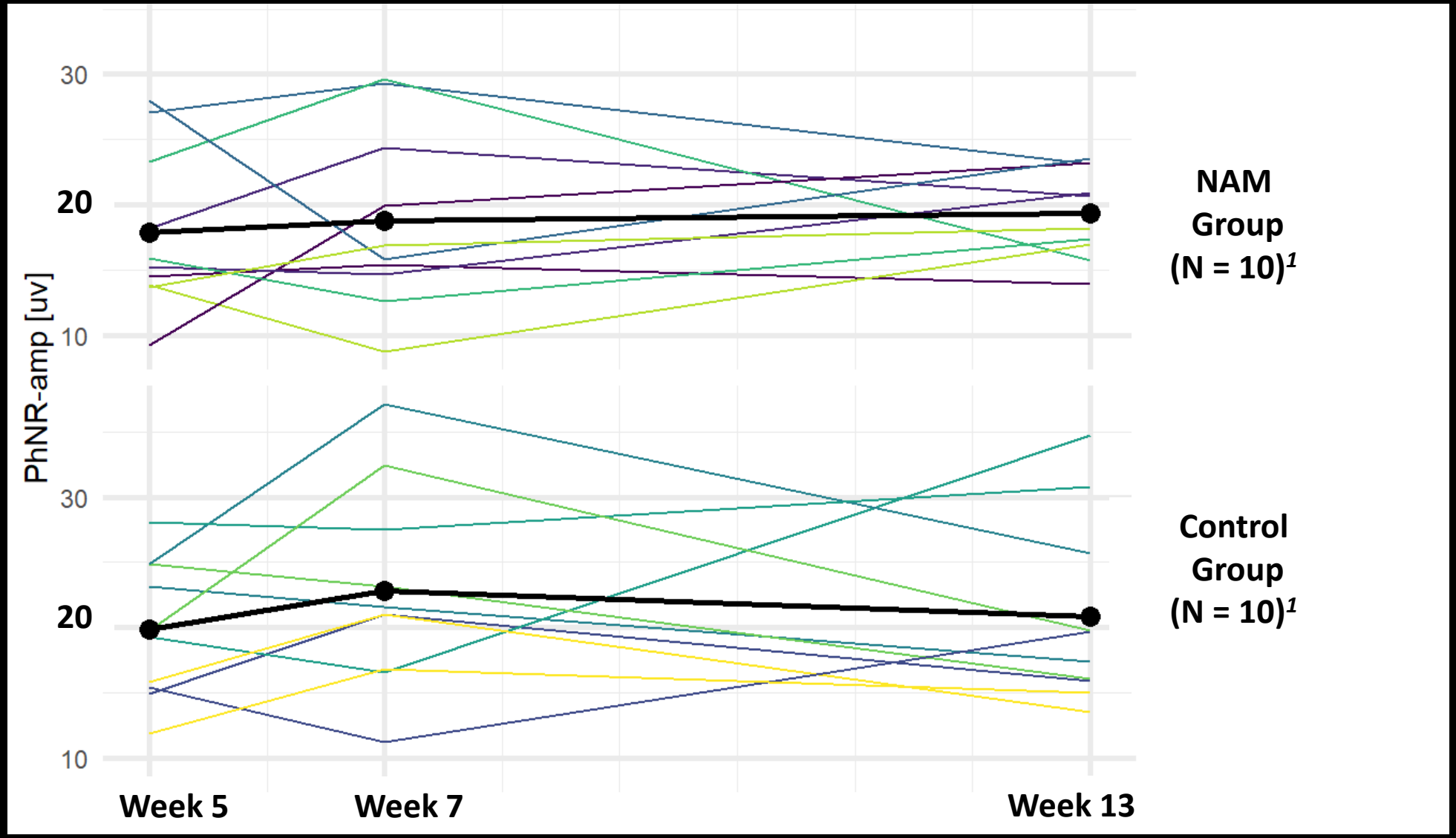
Variable	Week 5 Control Group (N = 10)[1]	Week 5 NAM Group (N = 10)[1]	Week 5 p-value[2]	Week 7 Control Group (N = 10)[1]	Week 7 NAM Group (N = 10)[1]	Week 7 p-value[2]	Week 13 Control Group (N = 10)[1]	Week 13 NAM Group (N = 10)[1]	Week 13 p-value[2]
b-time [ms]	31.78 (0.94)	32.17 (1.30)	0.69	30.50 (0.65)	31.93 (1.03)	0.001	31.77 (1.29)	31.54 (0.66)	0.97
b-amp [uv]	27.76 (9.36)	16.31 (6.39)	0.003	29.68 (6.74)	15.76 (5.37)	<0.001	26.58 (6.30)	20.29 (3.99)	0.026
PhNR-time [ms]	46.25 (2.34)	42.98 (2.66)	0.012	44.14 (2.12)	43.60 (3.66)	0.82	46.28 (2.24)	45.95 (1.83)	0.44
PhNR-amp [uv]	19.81 (5.31)	17.90 (6.22)	0.38	22.83 (7.72)	18.73 (6.99)	0.21	20.86 (7.18)	19.36 (3.40)	0.94
Ratio of PhNR-amp [uv] / b-amp [uv]	0.75 (0.18)	1.19 (0.36)	0.005	0.78 (0.22)	1.30 (0.60)	0.005	0.79 (0.19)	0.98 (0.22)	0.075

[1] Mean (SD)
[2] Wilcoxon rank sum test; Wilcoxon rank sum exact test

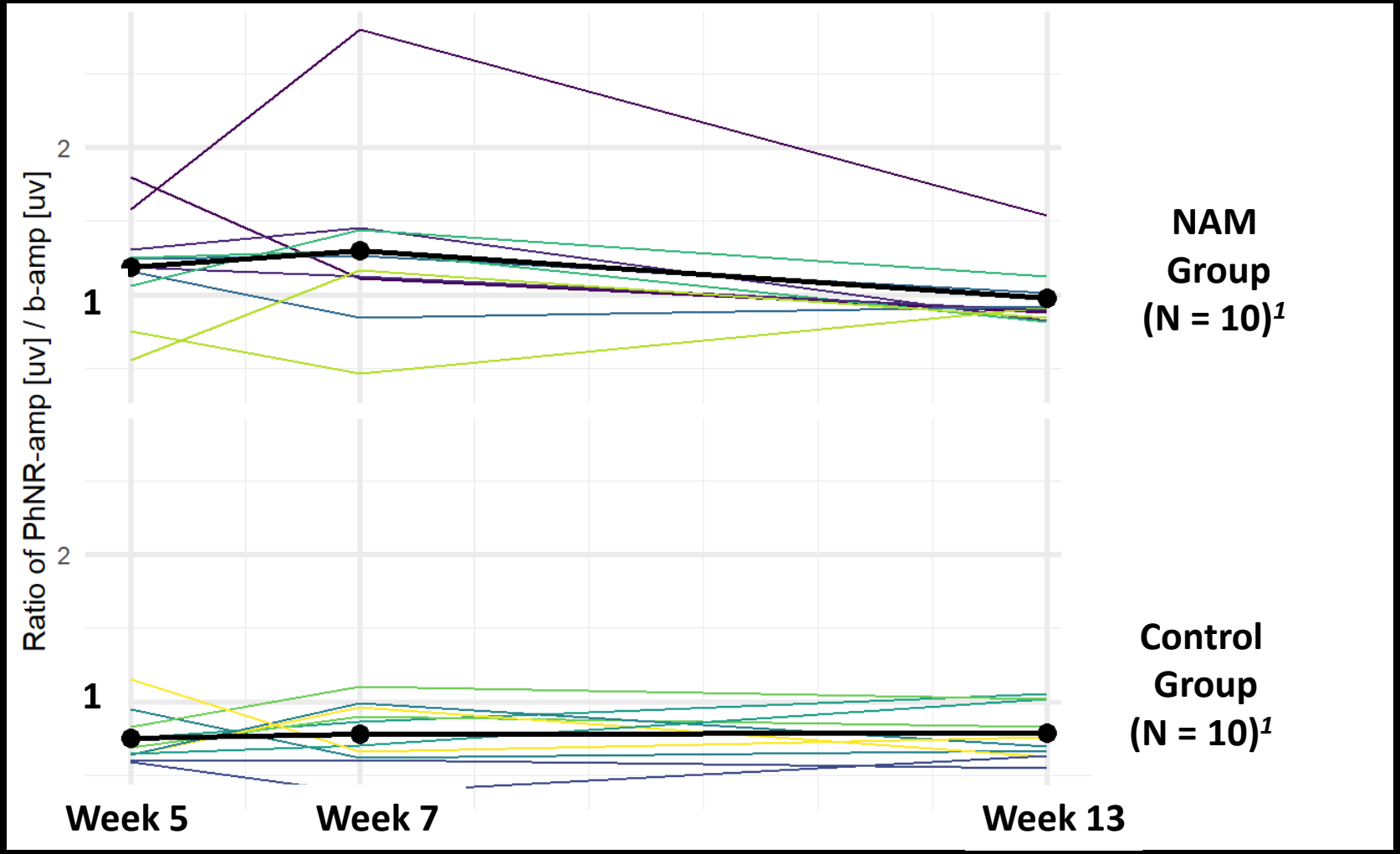
B-wave Amplitude Grouped by Treatment



PhNR Amplitude Grouped by Treatment



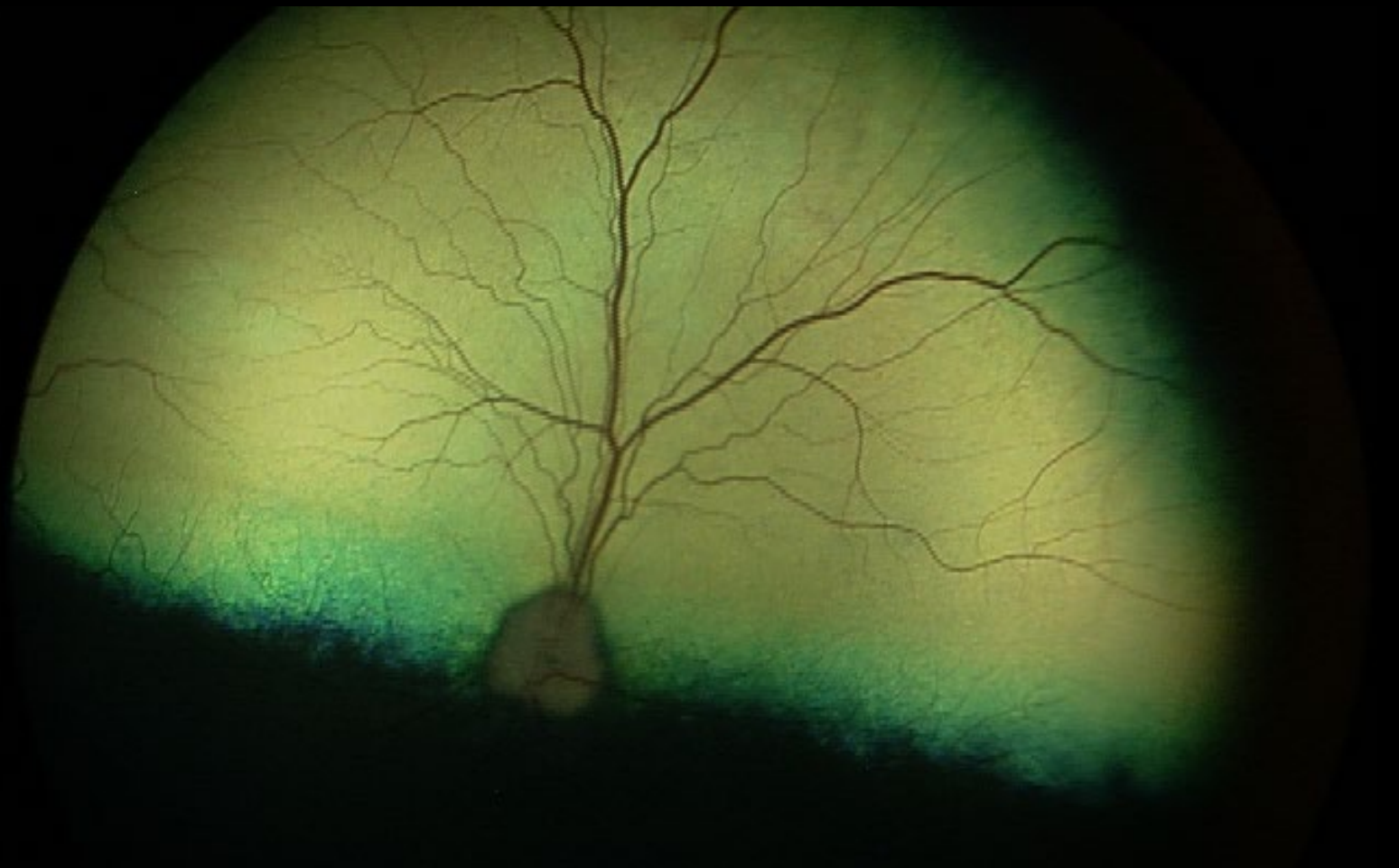
PhNR Amplitude / B-wave Amplitude Grouped by Treatment



Results – Ophthalmic Monitoring

Ophthalmic Monitoring

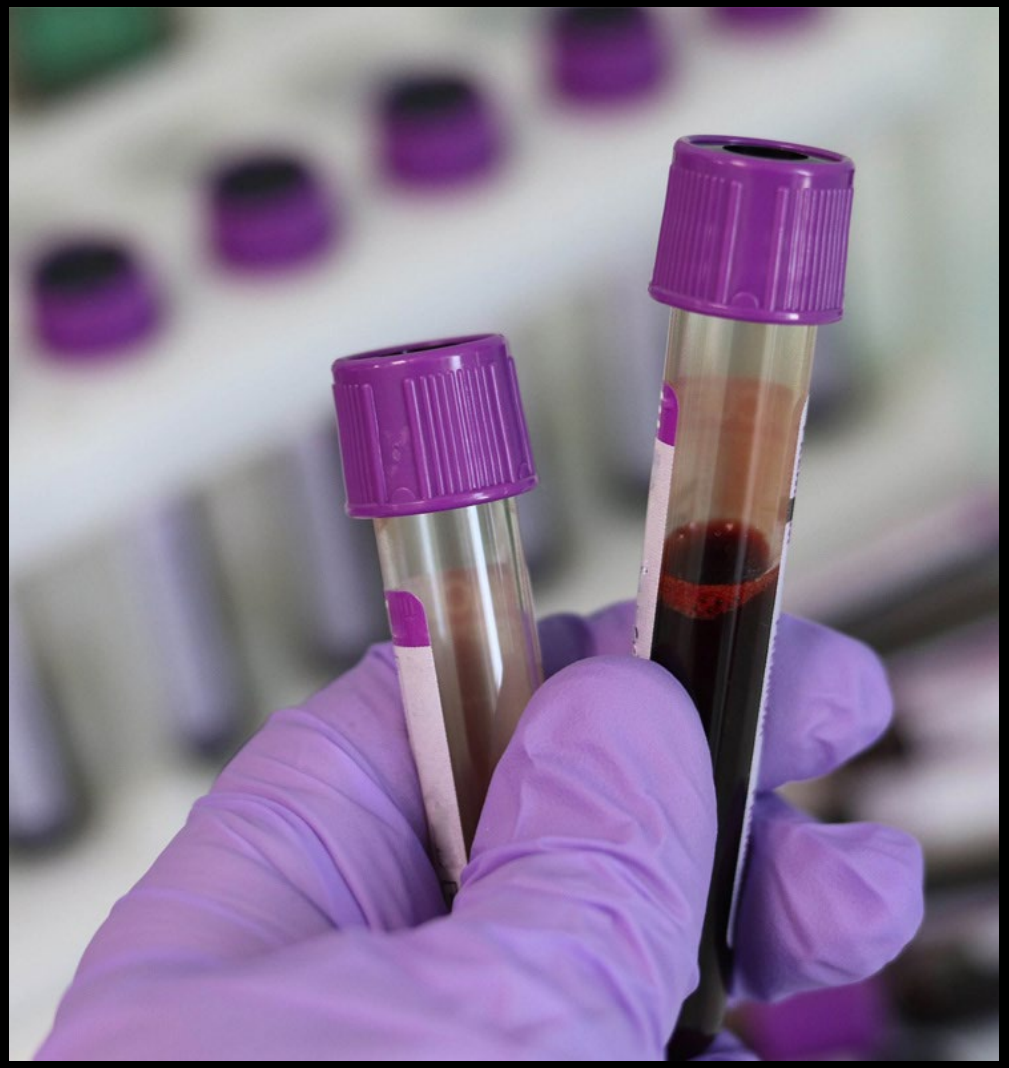
- Ophthalmic Exams
- Intraocular Pressure
  - Steady IOPs
- Retinal Photography



Results – Systemic Monitoring

Systemic Monitoring

- Bloodwork
  - CBC
  - Chemistry
  - Electrolytes
  - Lutein
- Physical Exams
  - General health
  - Weight



https://hospital.vetmed.wsu.edu/2022/01/12/understanding-your-pets-lab-tests/

Summary

- Baseline comparisons between groups shows the groups were similar
- Minimal differences between groups with respect to *time* (cone bipolar cells and retinal ganglion cells) and short-term supplementation
- Some statistical difference, but no clinical significance was found between groups with respect to *amplitude* (cone bipolar cells and retinal ganglion cells) and short-term supplementation

Short-term NAM supplementation at selected standard doses did not result in a noticeable improvement of inner retinal function in the well-established, clinically relevant large animal OAG model.

Limitations

- Small sample size
  - Each group with 5 dogs
- Short timeline
  - 5 weeks of supplementation
- ERG
  - Low flash intensities
  - Low electrical responses
  - Noise

Conclusion

- First study to evaluate effects of nicotinamide supplementation in canine POAG
- No detectable significant treatment effect of NAM for any o the tested ERG parameters were noted for short-term supplementation
- Nicotinamide could still be neuroprotective to prevent optic nerve degeneration when used for long-term treatment.

Conflict of Interest

- Dr. András Komáromy
  - CRISPR Therapeutics
  - PolyActiva Pty Ltd
  - Animal Necessity, LLC
  - Reichert, Inc.

Acknowledgements

- MSU – DVM Summer Research Program
- Project Funding
  - NIH R01-EYO25752
  - NIH R01-EY032478-01A1
  - BrightFocus Foundation
- Student Funding
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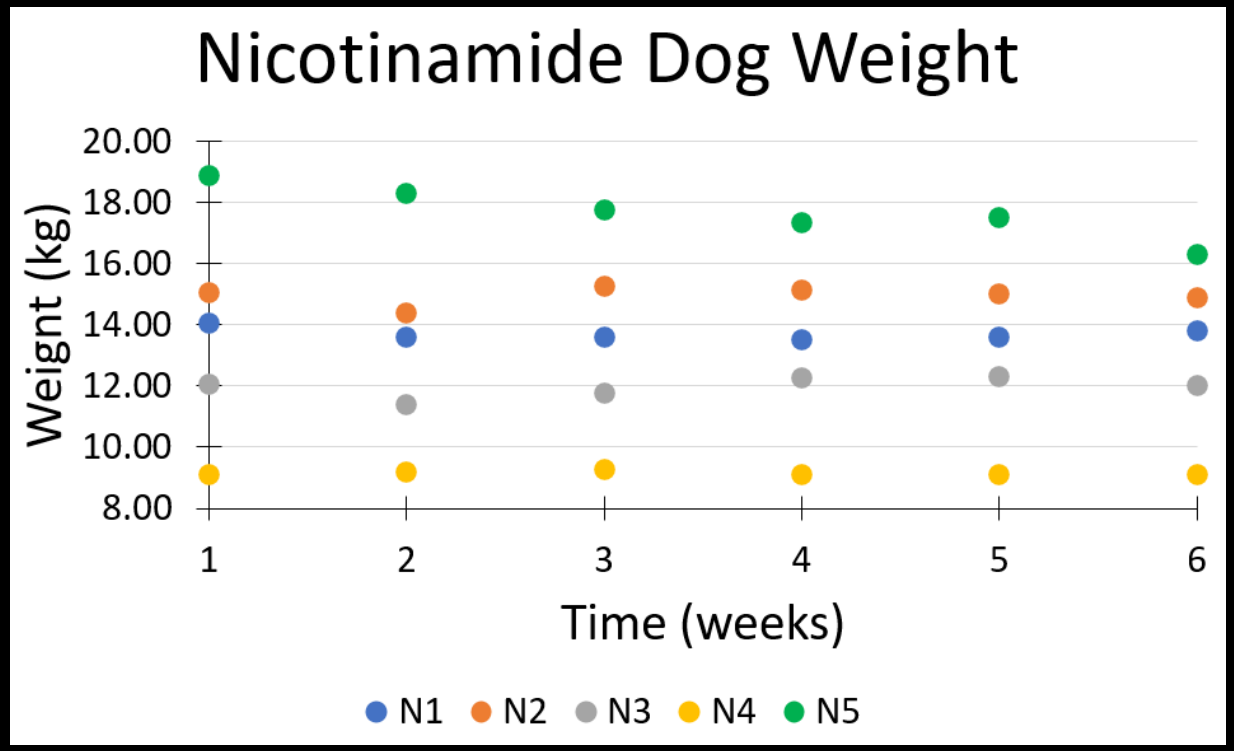
- Ophthalmology Mentorship
  - Dr. András Komáromy
- Komaromy Lab Director
  - Christine Harman
- Anesthesia
  - Mandy Anderson
- Statistics
  - Sichao Wang
- Vivarium
  - Kimberly Williamson
  - Ava Cabble
  - Lydia Kapeller
  - Cheyenne DeQuattro

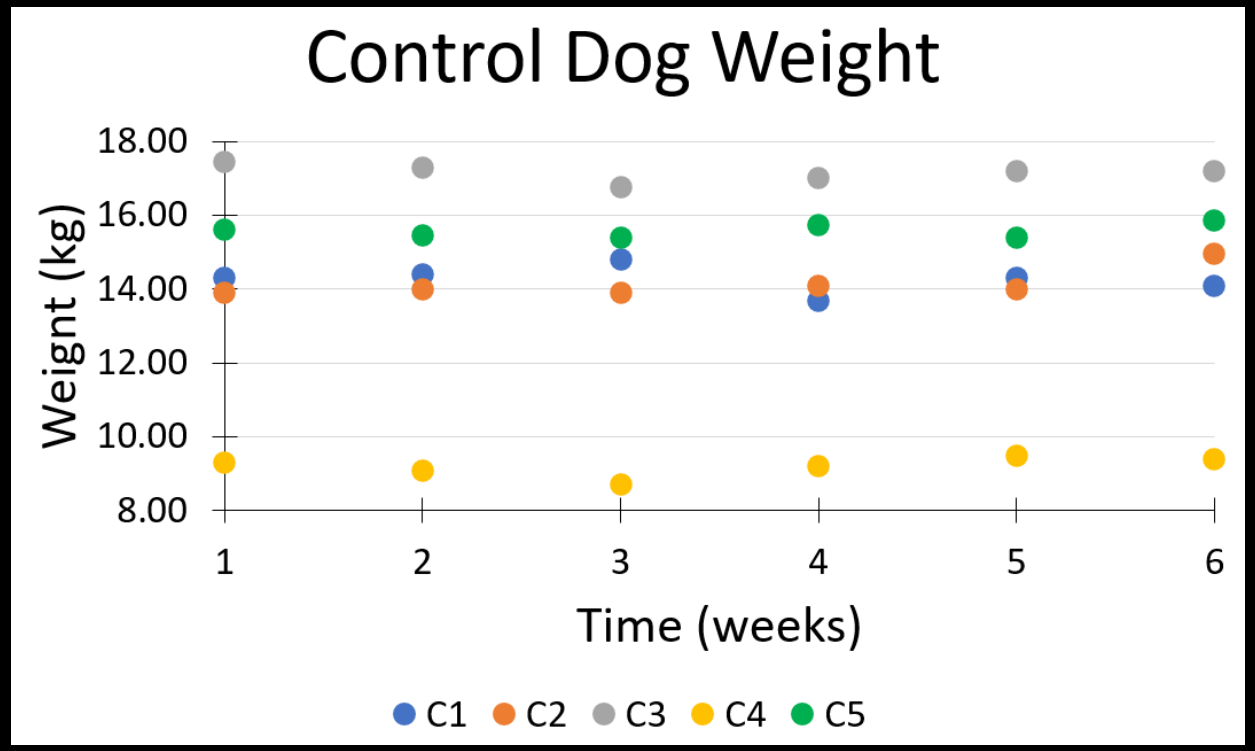
Left to right: Emily, Lydia, Ava, Cheyenne, Mandy, Christine, and András

QUESTIONS

EXTRA SLIDES

Dose & Dosage Calculations



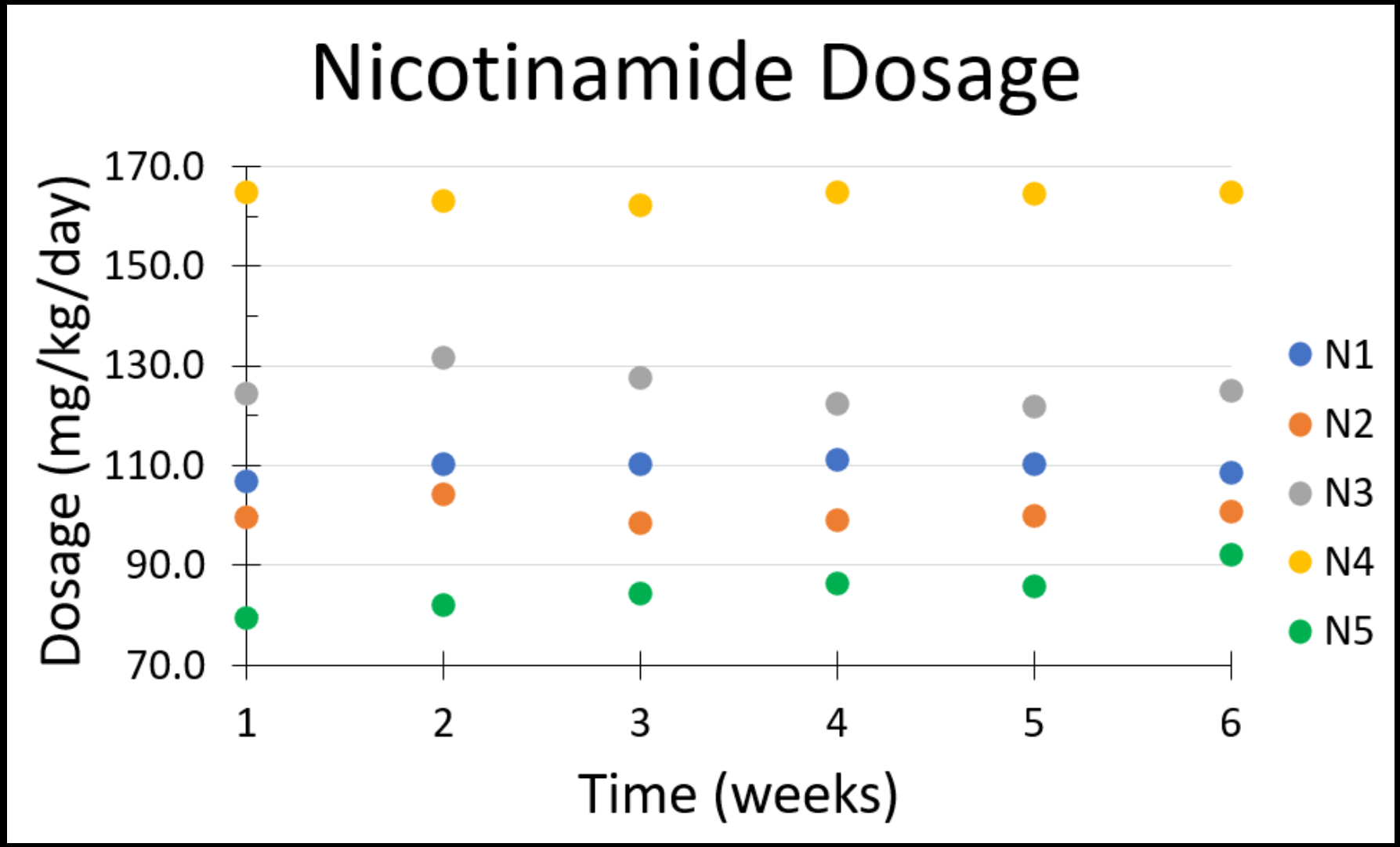


Nicotinamide Group:
Dose: 1,500 mg Nicotinamide/day

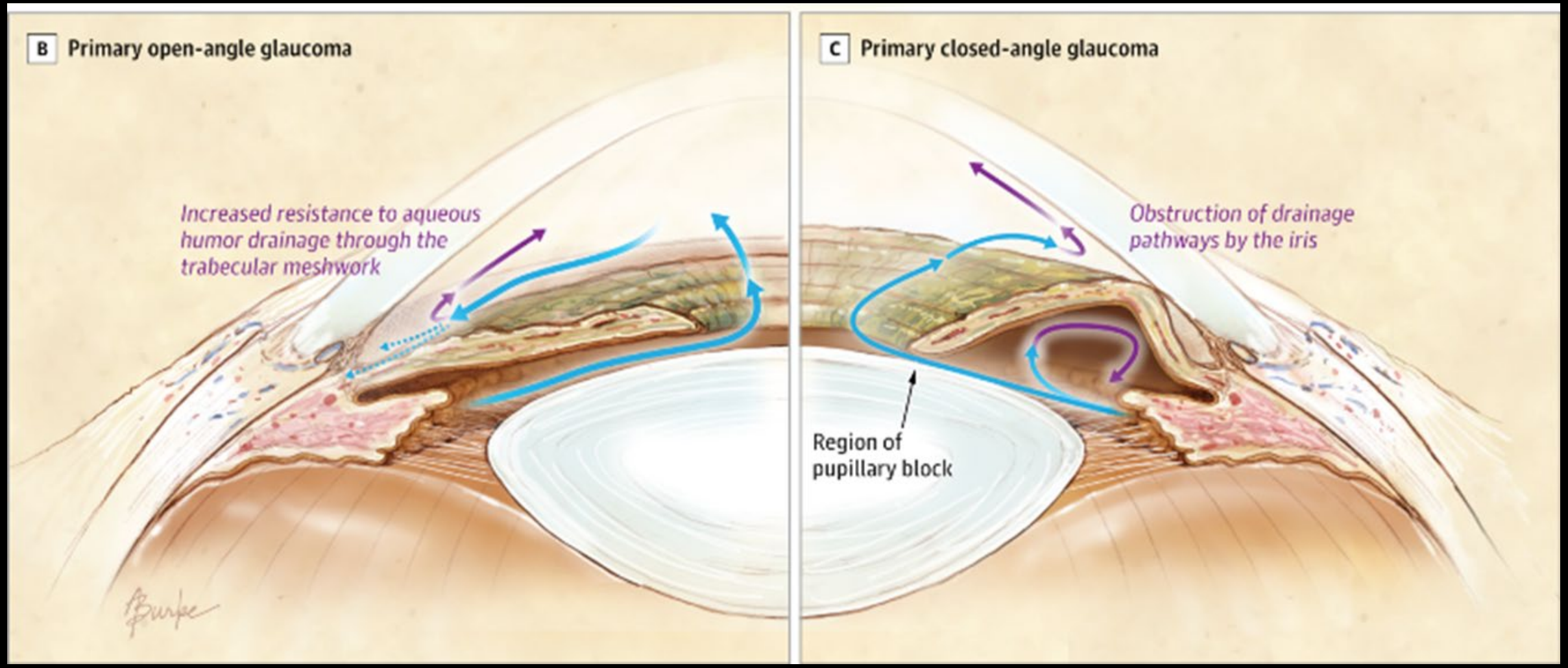
Control Group:
Dose: no supplementation

Dosage

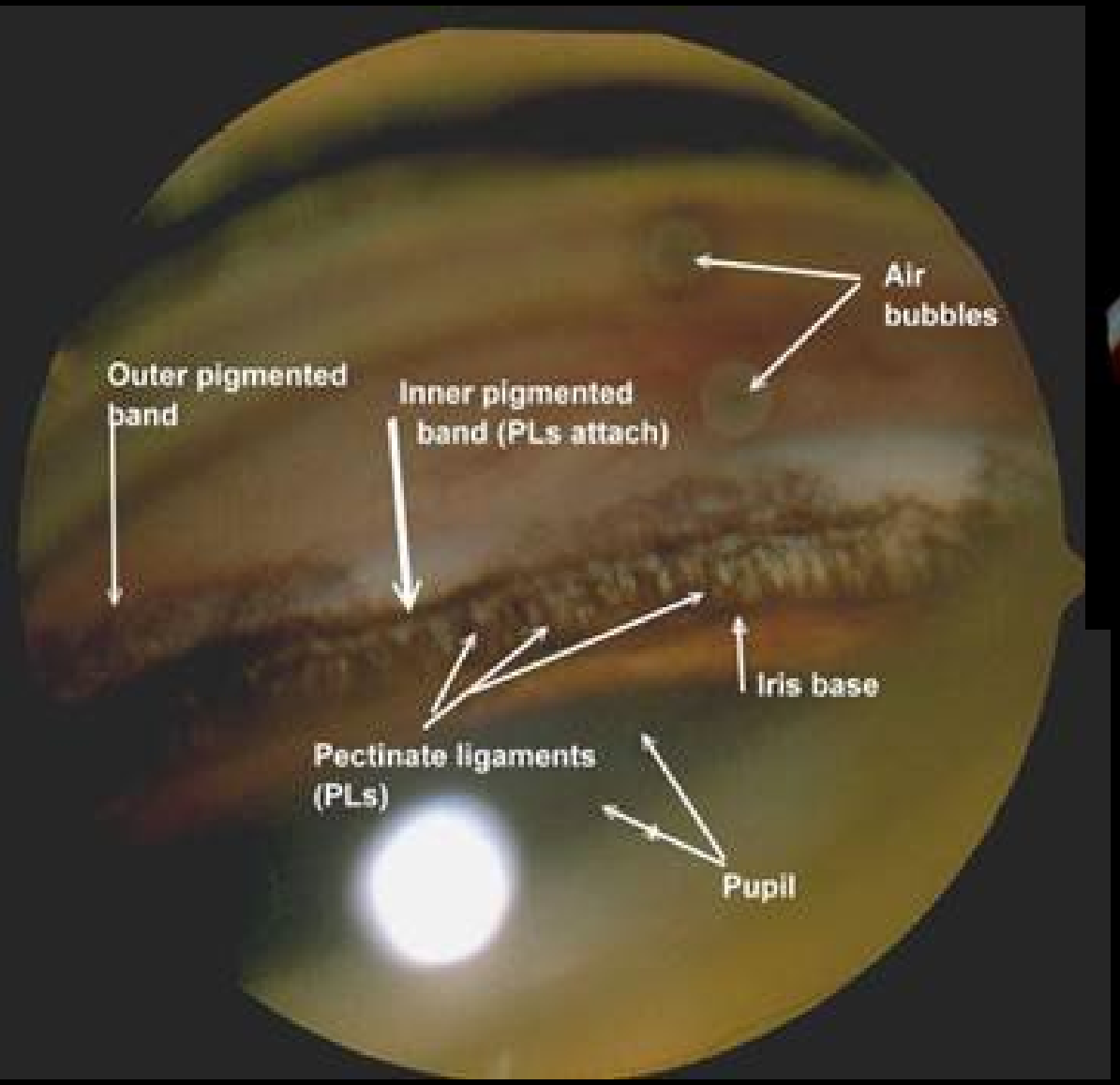
N5 lost weight and increased dosage

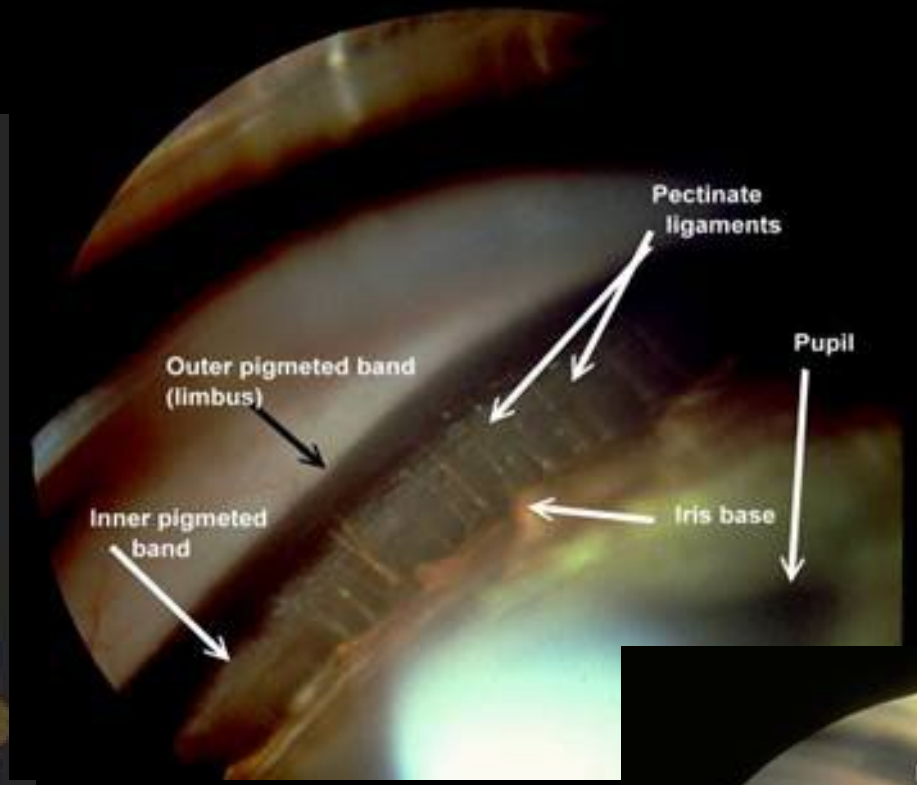


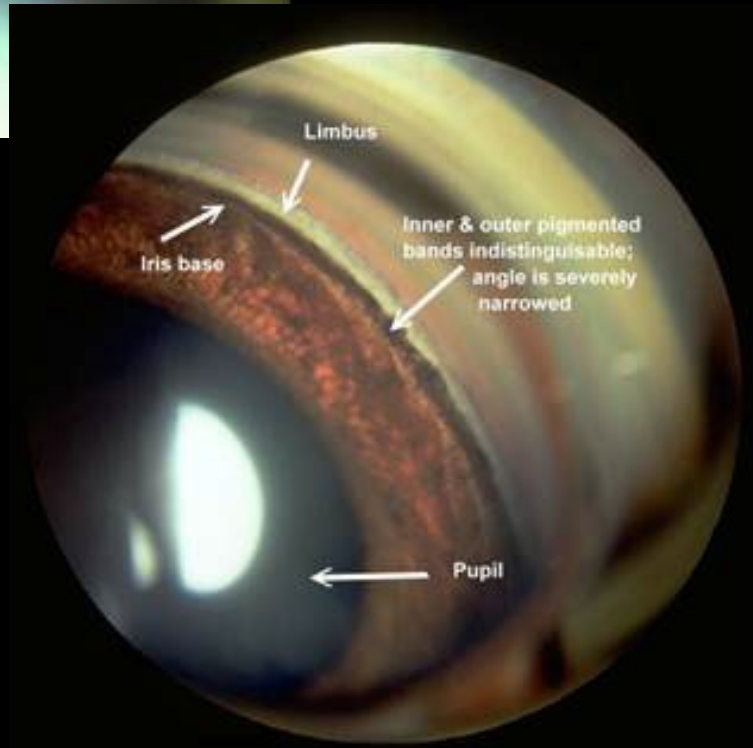
`Open-Angle' Glaucoma



Kuchtey et al. 2011







https://eyedvm.com/animal-eye-care/handout/glaucoma-in-dogs-and-cats/

Materials & Methods

Nicotinamide Supplementation

- Treatments sheets
- Instructions
- Accountability

Regular Diet Information

Materials & Methods

Meatballs

- Purina Pro Plan, Veterinary Rx Diet, HA Hydrolyzed

  (chicken flavor)

Materials & Methods

Nicotinamide Supplementation

- 1 capsule = 500 mg Nicotinamide

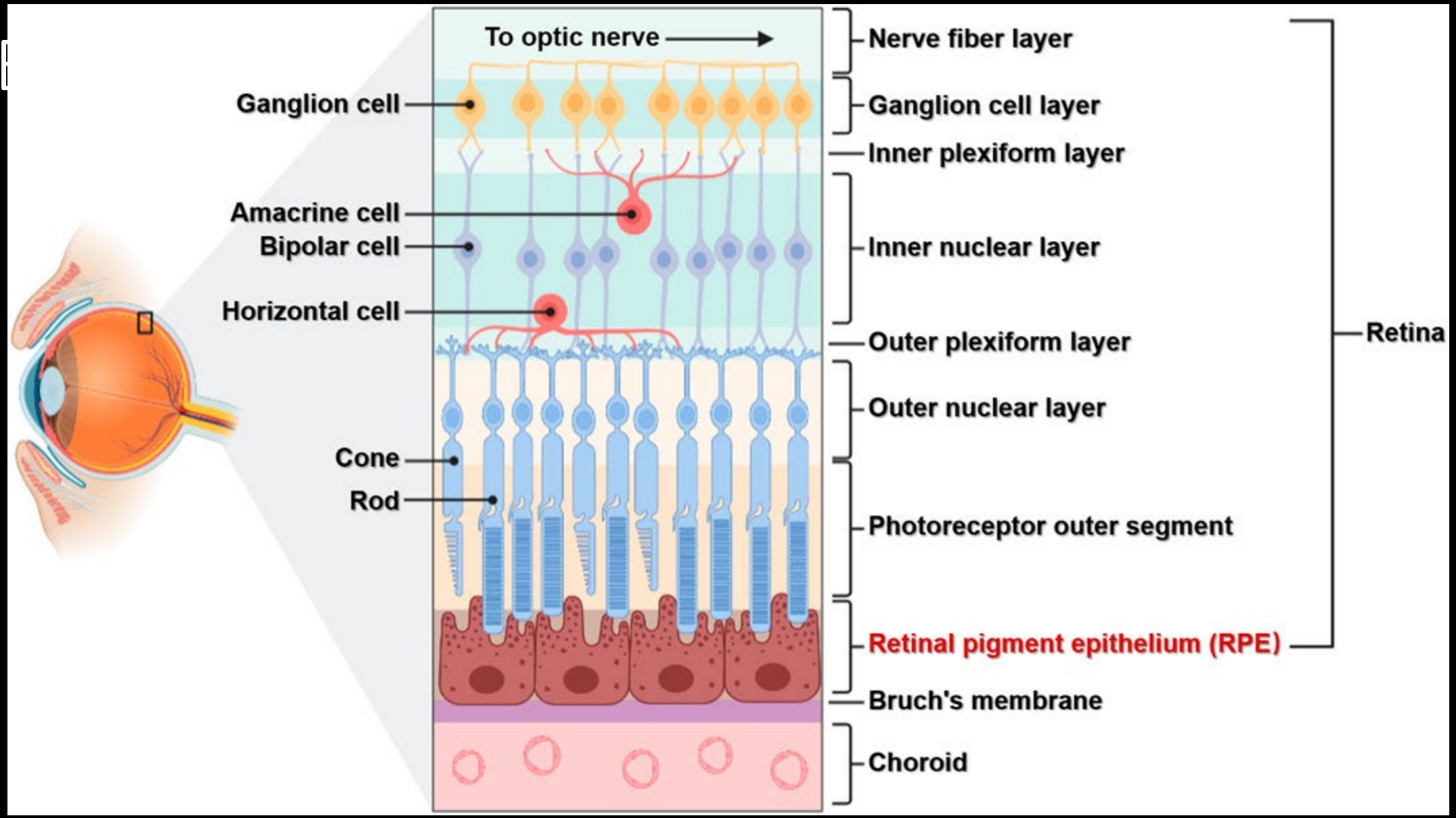
- Purina Pro Plan, Veterinary Rx Diet, HA Hydrolyzed

  (chicken flavor)

Materials & Methods

Retinal Photography

Kuchtey et al. 2011


To optic nerve
Ganglion cell
Amacrine cell
Bipolar cell
Horizontal cell
Cone
Rod
Nerve fiber layer
Ganglion cell layer
Inner plexiform layer
Inner nuclear layer
Outer plexiform layer
Outer nuclear layer
Photoreceptor outer segment
Retinal pigment epithelium (RPE)
Bruch's membrane
Choroid
Retina

Materials & Methods

Retina Function

- Electroretinography (ERG)
  - Red on Blue
    - Blue light adapted (5 minutes)
    - Red flash
      - 0.07
      - 0.90
      - 1.74
      - 2.57
      - 3.40
      - 4.00

Anasthesia

- 0.5 mg/kg Ondansetron tablets
- Pre-meds
  - 0.02 mg/kg Acepromazine and 0.2 mg/kg Butrophanol given intravenously
- Induction
  - 30-50 mg of Midazolam was given intravenously based on age
  - and 4-7 mg/kg propofol was given to effect.
  - Crystalloid maintenance fluids given 10-12 mg/kg/hr while under anesthesia.
- Maintenance
  - with 1-2% isoflurane
  - 1-1.5 L/kg/min oxygen flow rate with house oxygen and passive scavenging with activated charcoal canisters.

Dorzolamide / Timolol

- Cosopt (dorzolamide / timolol)
  - **Dorzolamide** → carbonic anhydrase inhibitor
    - Decreases aqueous humor production 30-60%
  - T**imolol** → beta blocker
    - Beta 1 and beta 2 blockade in ciliary process
  - Effect: lower eye pressure by lowering the amount of fluid in the eye

  - Eye Dropper

    <u>10ml of 2%/0.5%</u>

ERG	7 – weeks NAM Group Mean ± SD	7 – weeks Control Group Mean ± SD
b-wave amplitude (µV)	15.8 ± 5.4	29.7 ± 6.7
b-wave implicit time (ms)	31.9 ± 1.0	30.5 ± 0.7
PhNR amplitude (µV)	18.7 ± 7.0	22.8 ± 7.7
PhNR implicit time (ms)	43.6 ± 3.7	44.1 ± 2.1
PhNR/b-wave amplitude ratio	1.3 ± 0.6	0.8 ± 0.2

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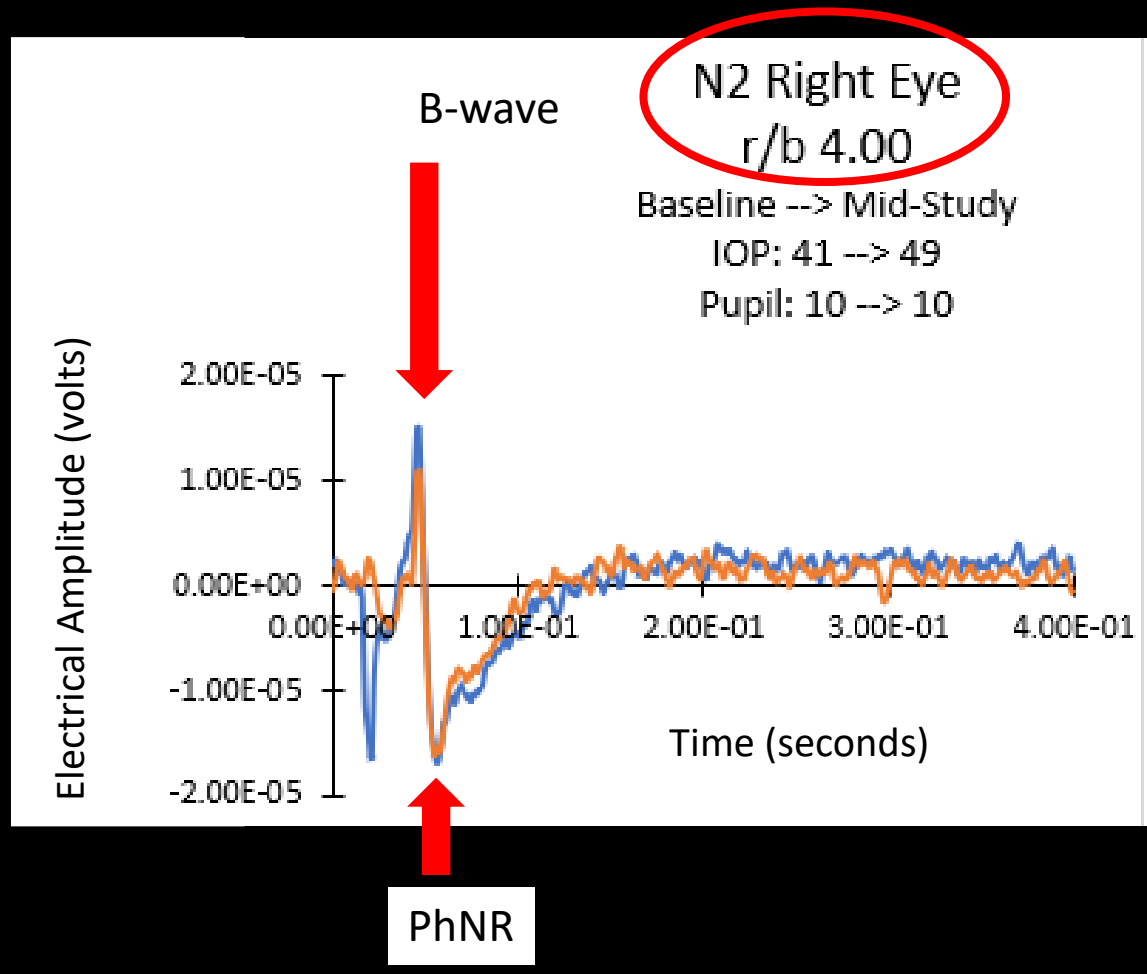
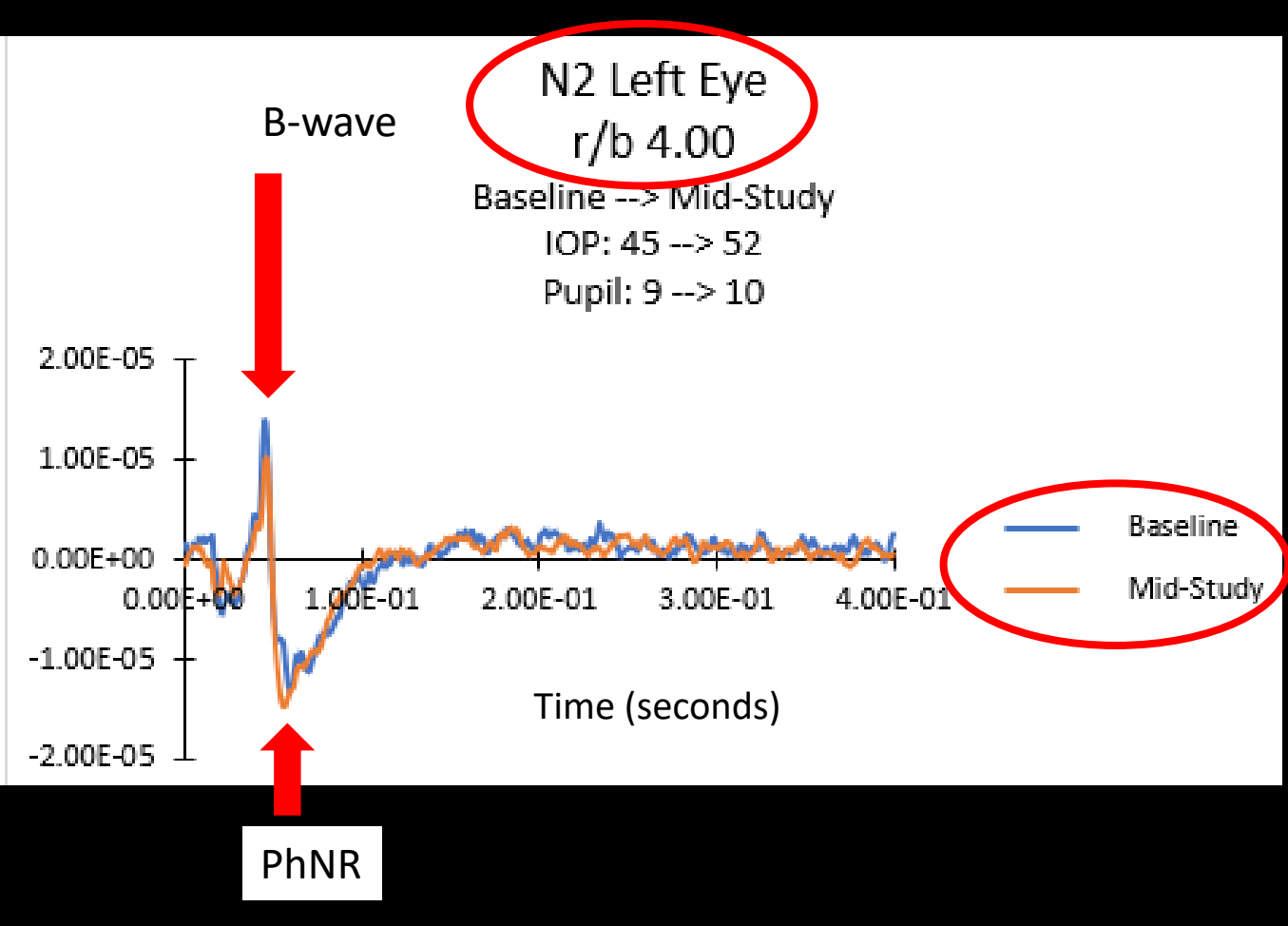
PhNR implicit time (ms)	43.6 ± 3.7	44.1 ± 2.1
PhNR/b-wave amplitude ratio	1.3 ± 0.6	0.8 ± 0.2

Results – Nicotinamide (Baseline to 5th week)

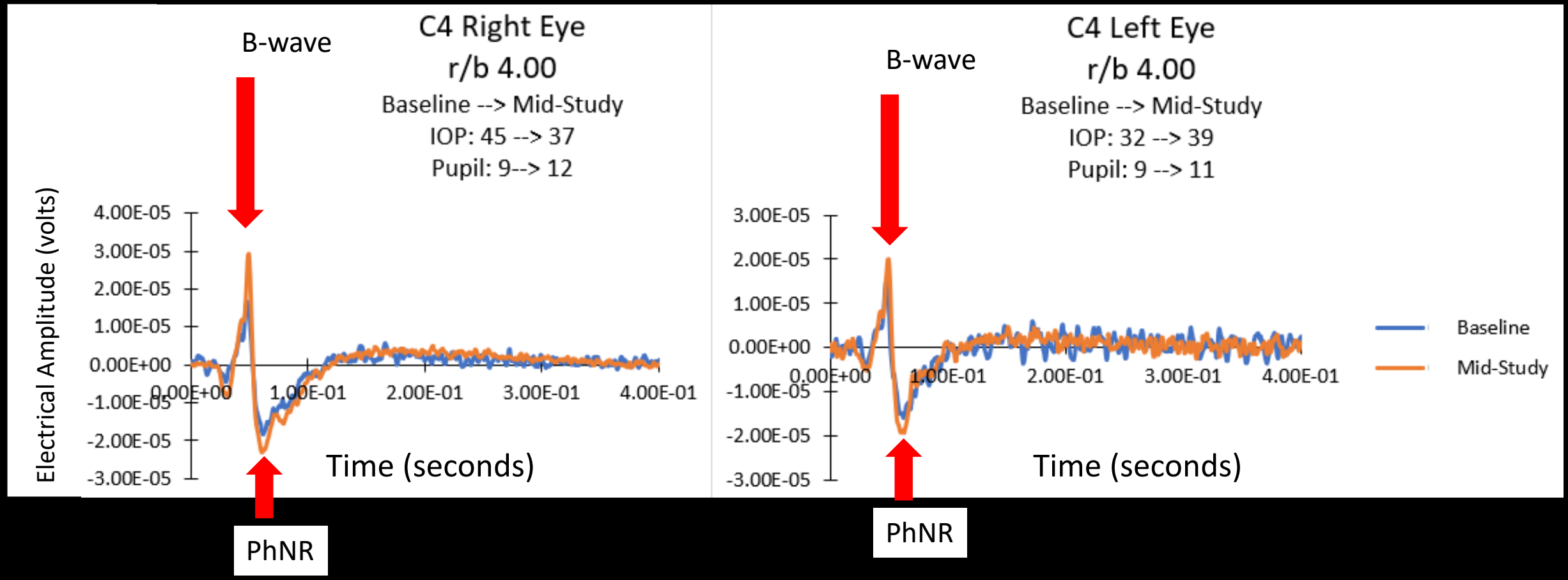
3 of 5 dogs *may* have had decreased rod bipolar cell function.





Results - Sham (Baseline to 5th week)

B-wave: 8 of 10 eyes *may* have increased rod bipolar cell function.



PhNR: 9 of 10 eyes *may* have increased ganglion cell function.

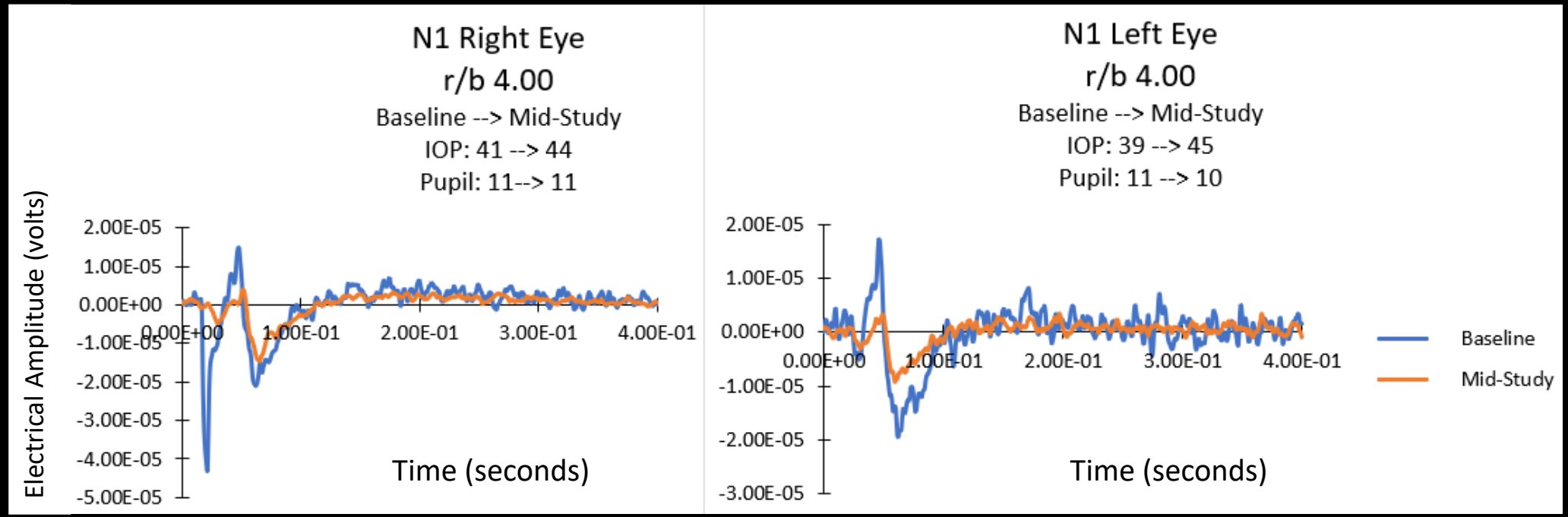
Results

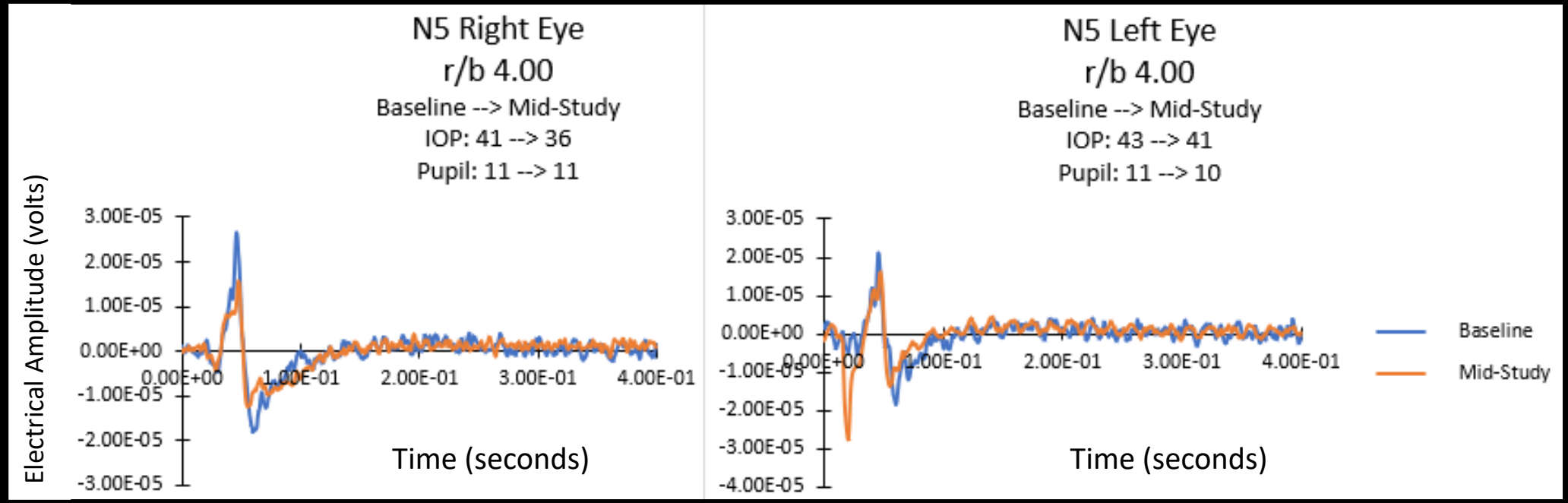
N1

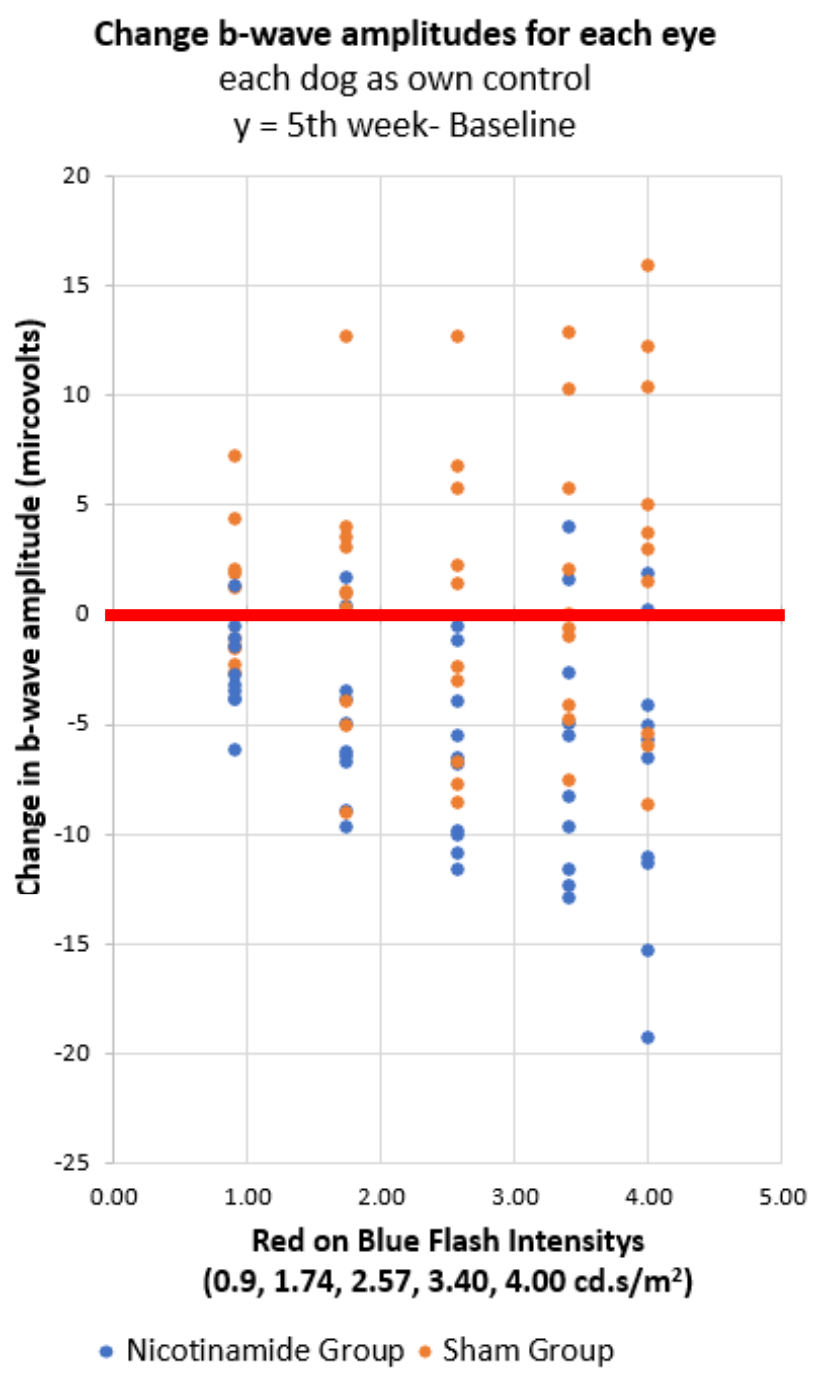
- Oldest (6.4 yrs)
- Dorzolamide / Timolol

N5

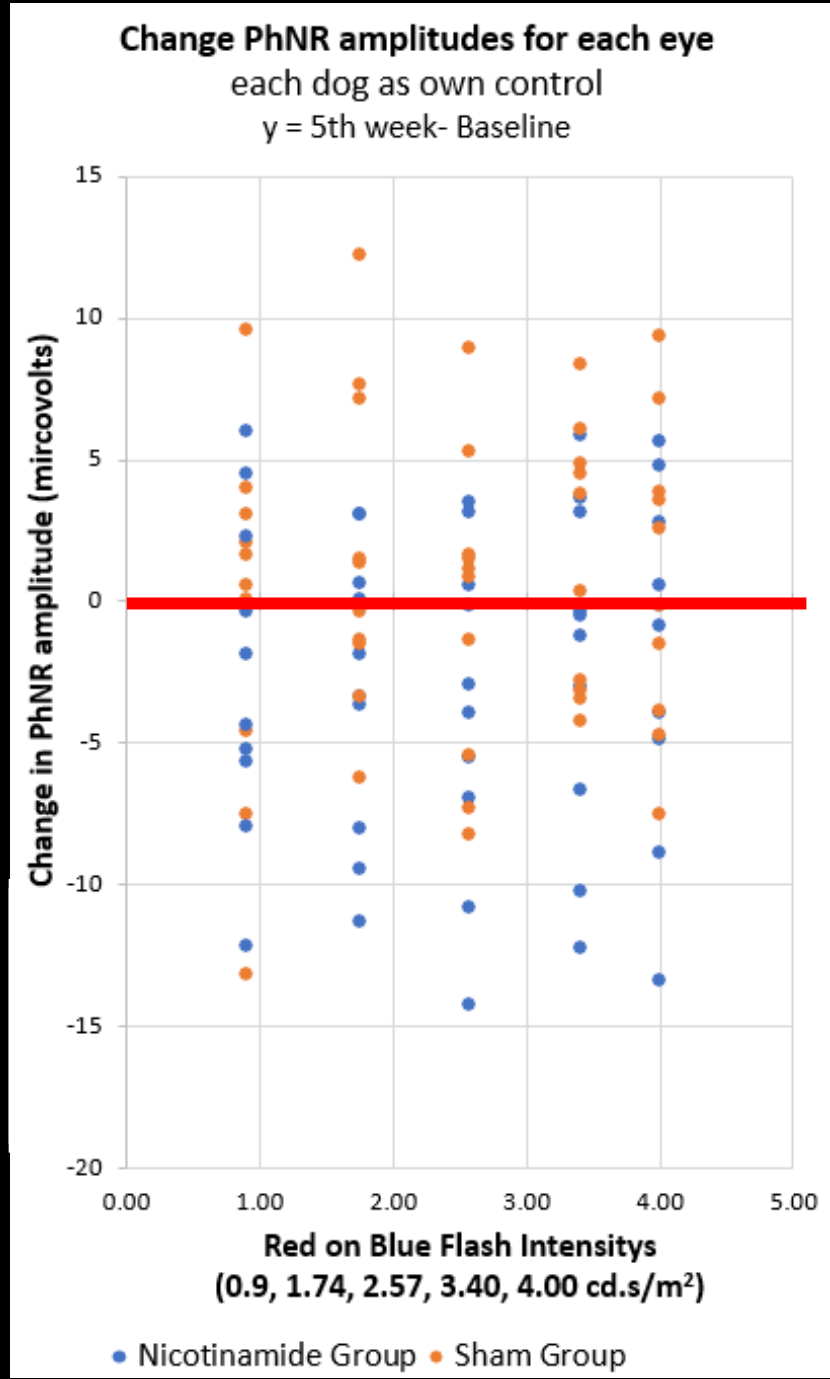
- Dorzolamide / Timolol
- more...





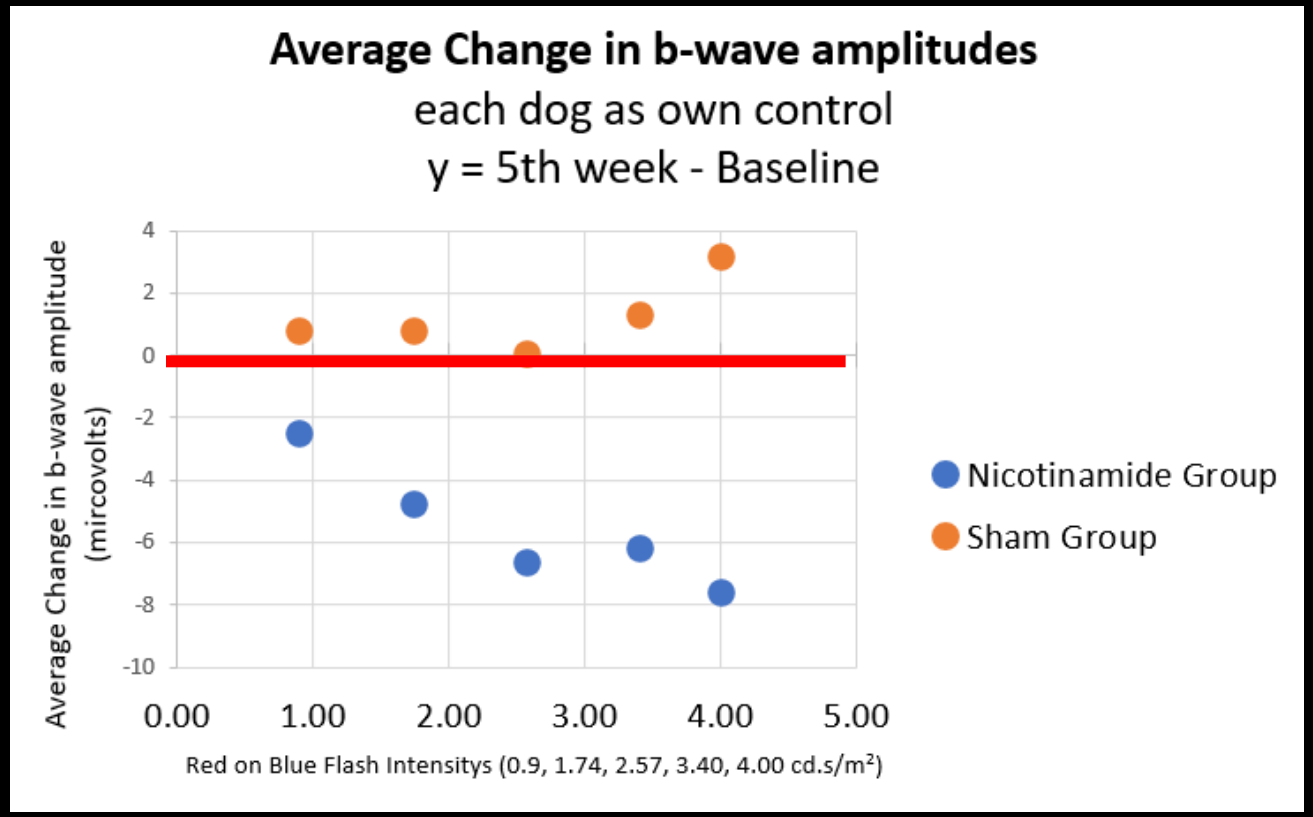


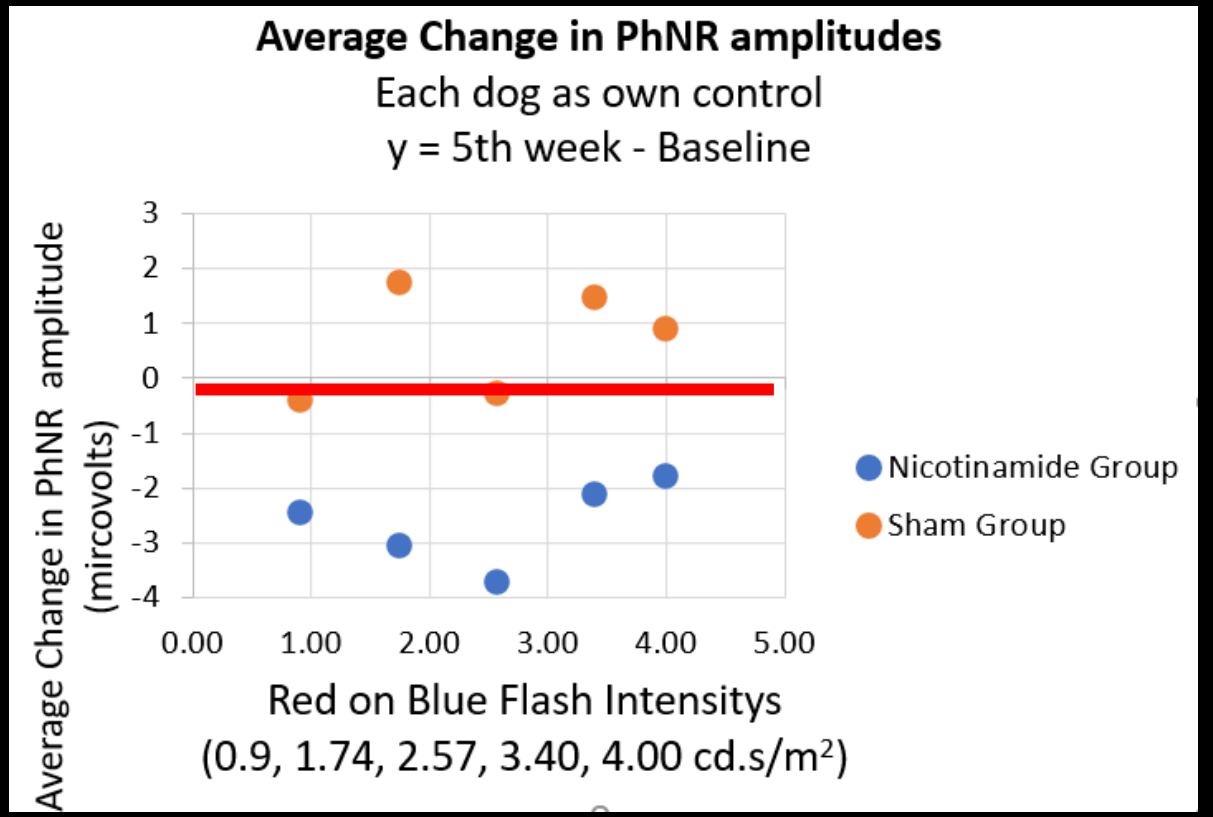
Change in each Individual Eye



Results – Average Change in Group Amplitudes

B-wave: the sham group *may* have increased rod bipolar cell function compared to nicotinamide group.





Plumb's

- Dose recommendations
- Monitoring recommendations
  - Liver enzymes; especially with,
    - Doxycycline
    - minocycline

Dosages

- **DOGS:**

  Adjunctive treatment of sterile inflammatory diseases (extra-label): Niacinamide may be used alone; however, more therapeutic benefit is seen in combination with a tetracycline. Recommended dosages are empirical and vary somewhat. Practically, for dogs weighing 5 kg or less: niacinamide 125 mg per dog PO 3 times a day; for dogs weighing 5-10 kg: niacinamide 250 mg per dog PO 3 times a day; for dogs weighing more than 10 kg: niacinamide 500 mg per dog PO 3 times a day. If efficacious, may reduce niacinamide dose to twice daily administration and then tapered further over time when possible.

  NOTE: When using doxycycline, recommended dose is 5 to 10 mg/kg PO twice daily. If substituting with minocycline, use 7.5 mg/kg PO twice daily.

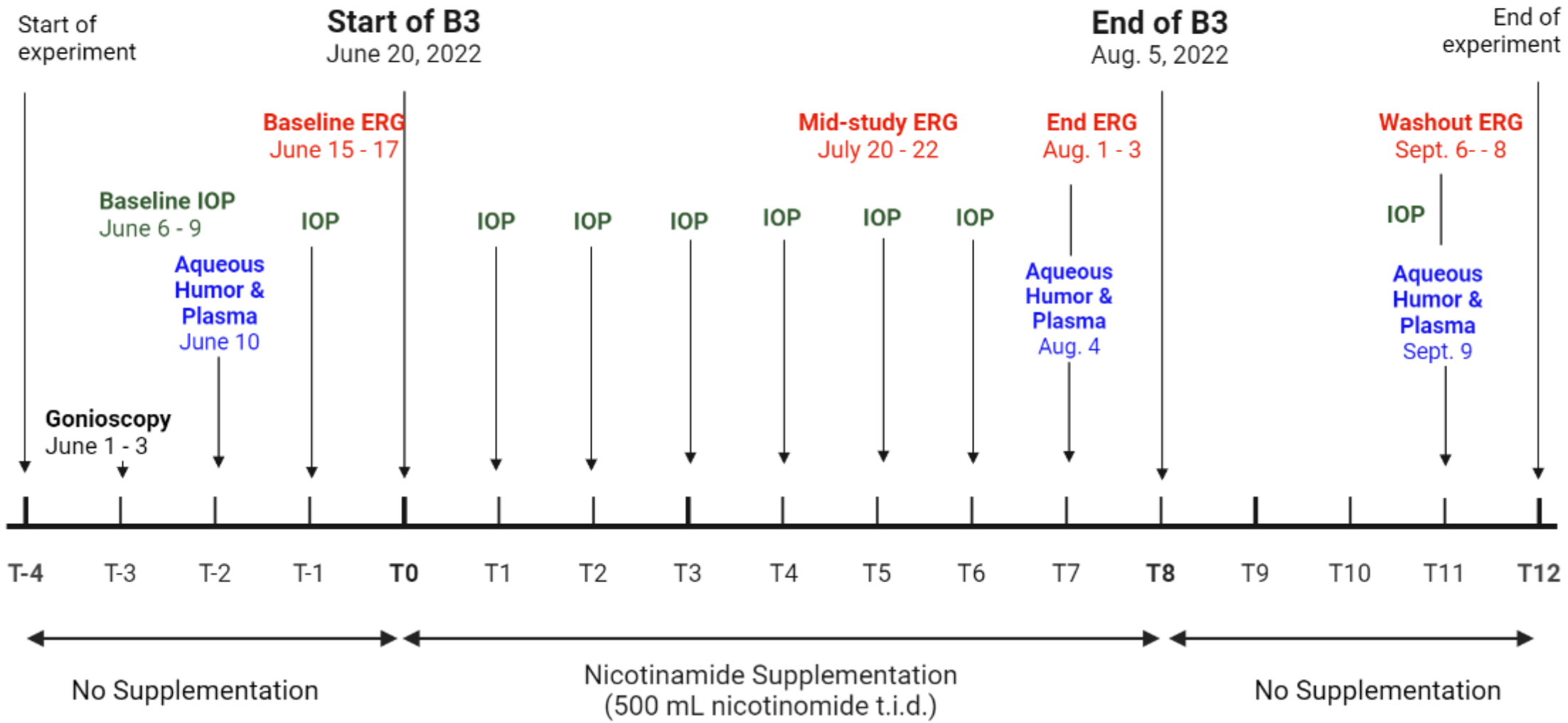
Monitoring

- Efficacy.
- Adverse effects (baseline and occasional monitoring of liver enzymes is suggested), particularly when combined with other drugs that may increase liver enzymes such as doxycycline or minocycline.

Client Information

- Used in dogs in combination with a tetracycline for treatment of a variety of serious skin and other autoimmune diseases.

Nicotinomide Study Timeline



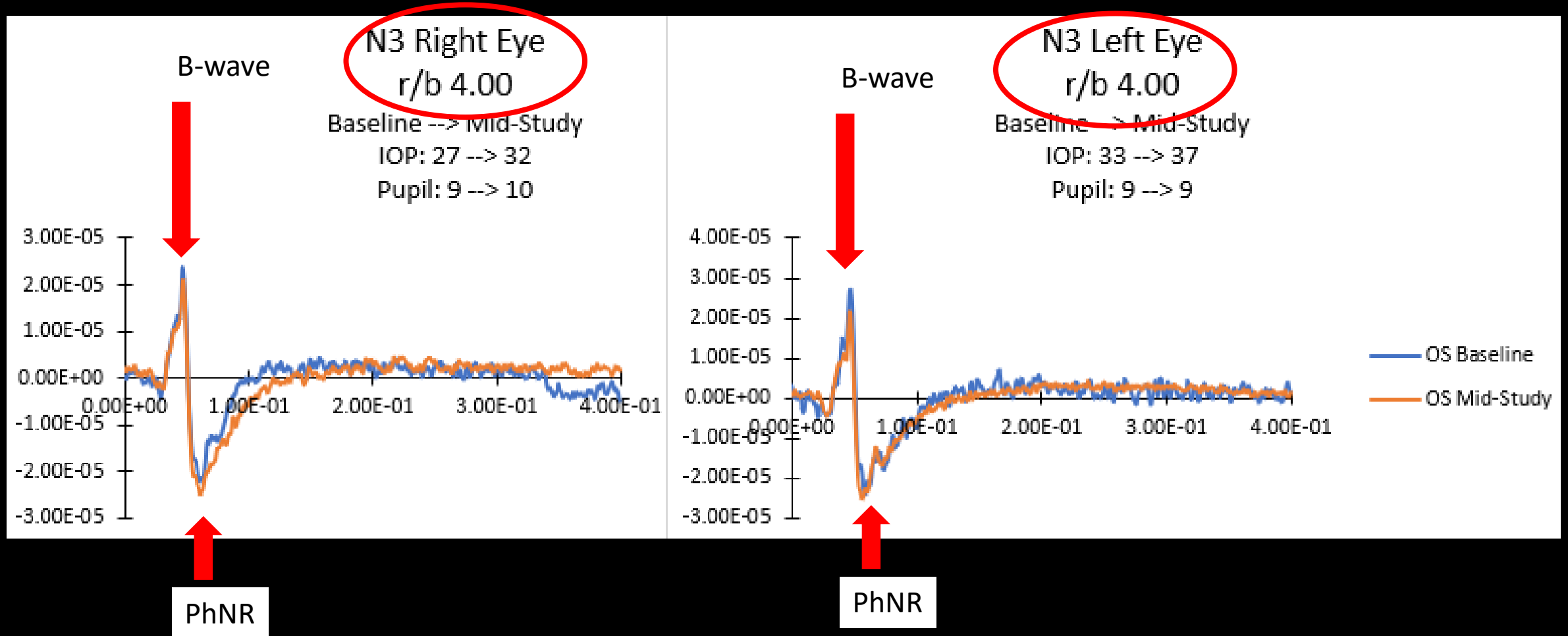
Materials & Methods

- Michigan State University
- College of Veterinary Medicine
- Komaromy Laboratory

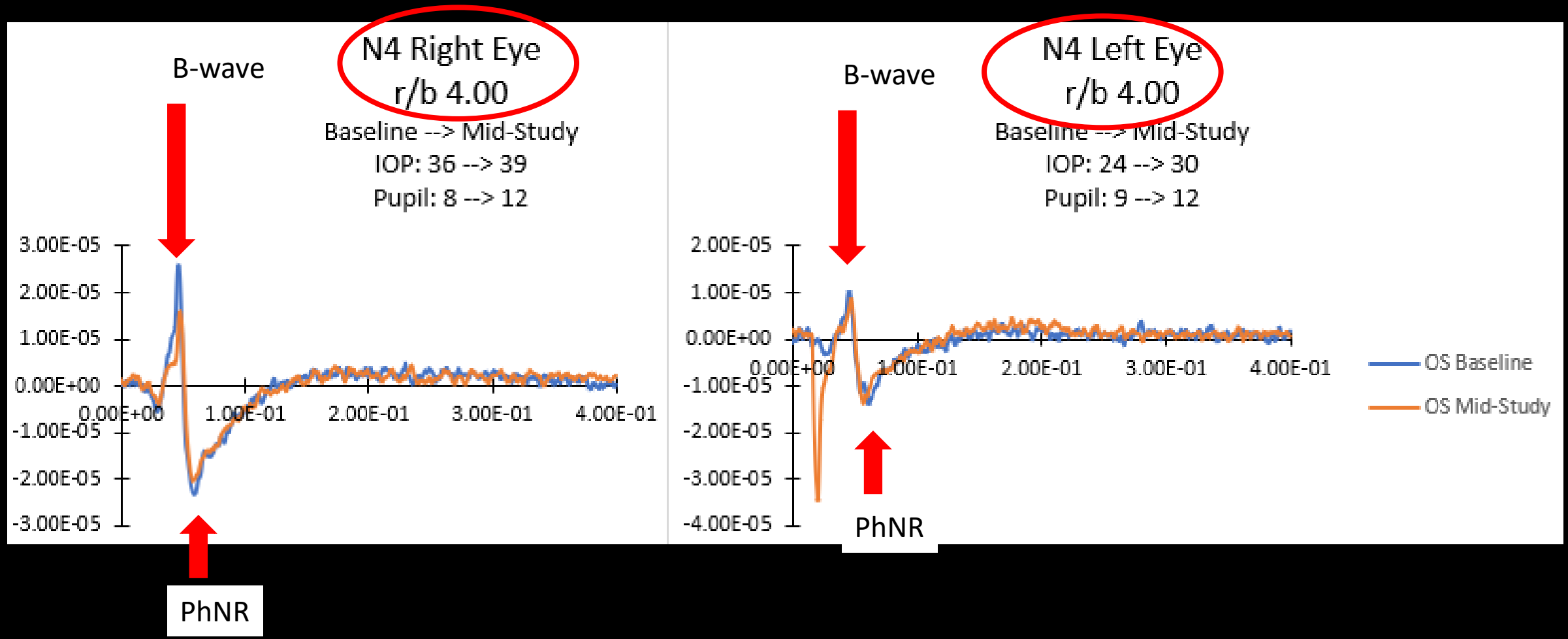
Results - Population

	Nicotinamide Group (n = 5) Mean ± SD Range	Sham Group (n = 5) Mean ± SD Range
Age **(years)**	4.7 ± 1.0 4.1 – 6.6	4.2 ± 0.2 4.1 – 4.4
Weight **(kg)**	13.5 ± 3.0 9.1 – 18.9	14.0 ± 2.7 8.7 – 17.5
Sex **(Male : Female)**	2 : 3	2 : 3

Results - Nicotinamide (Baseline to 5th week)

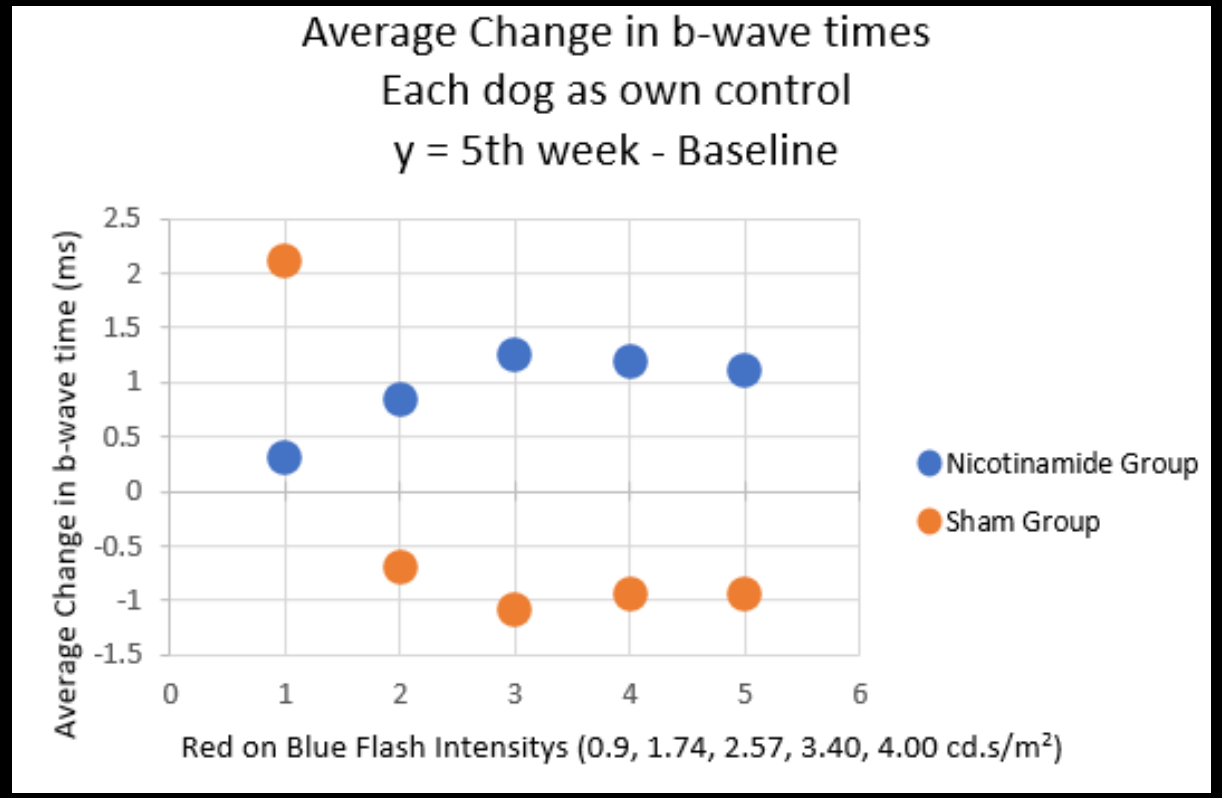


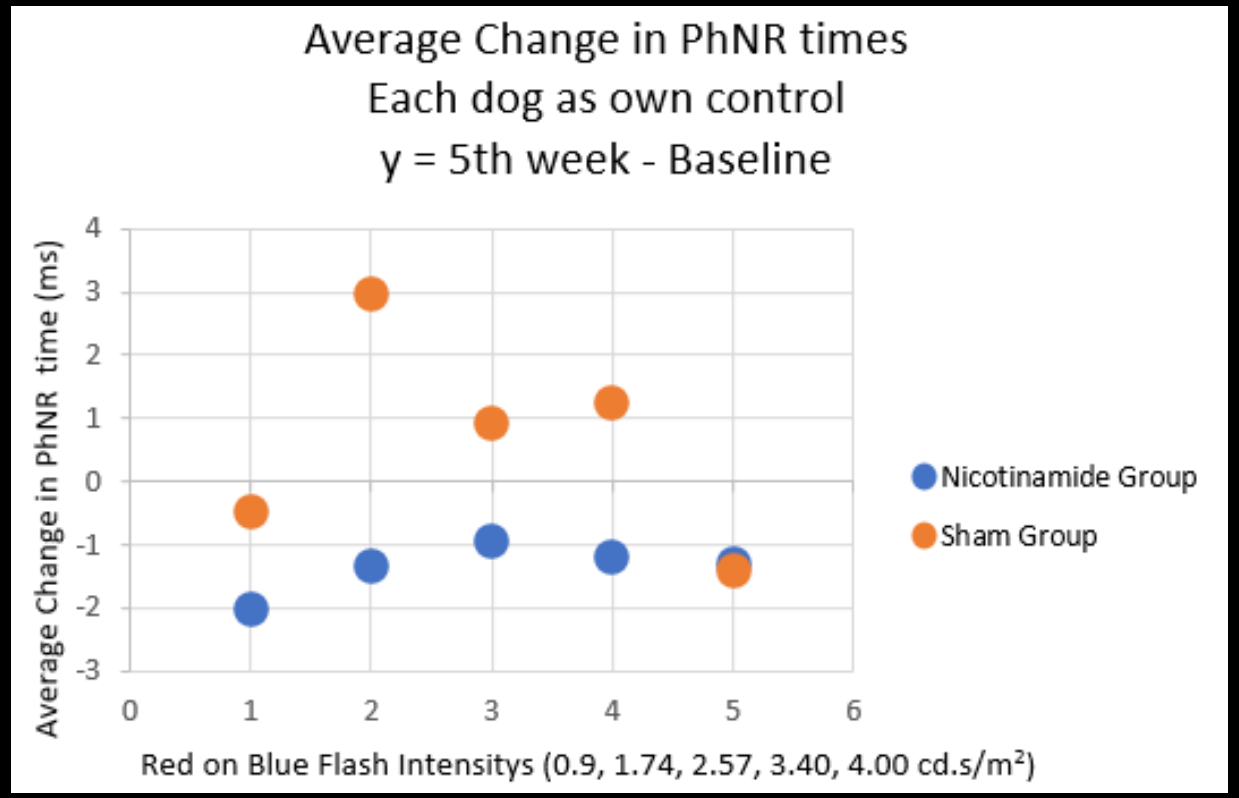
Results - Nicotinamide (Baseline to 5th week)



Results – Average Change in Group Times

B-wave: 8 of 10 eyes *may* have increased rod bipolar cell function.





Extras>>>>>>