Short-term Improvement in Inner Retinal Function with Nicotinamide (Vitamin B3) Supplementation in Glaucoma

A Crossover Randomized Clinical Trial

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Running head: Short-term improvement with vitamin B3 in glaucoma

Abbreviations and Acronyms: ATP – adenosine triphosphate, CI – confidence interval, ERG – electroretinogram, IOL – intraocular lens, IOP – intraocular pressure, IQR – interquartile range, MD – mean deviation, NAD^+ – nicotinamide adenine dinucleotide, NAM – nicotinamide, NTG – normal tension glaucoma, OAG – open-angle glaucoma, PDG – pigment dispersion glaucoma, PL – placebo, PXFG – pseudoexfoliative glaucoma, PhNR – photopic negative response, PSD – pattern standard deviation, Q1 – first quartile, Q3 – third quartile, RGC – retinal ganglion cells, RNFL – retinal nerve fibre layer, SD – standard deviation, SD-OCT – spectral domain optical coherence tomography, SEM – standard error of the mean, VF – visual field, Vmax – saturating amplitude

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Precis: First clinical investigation in nicotinamide (vitamin B3) supplementation demonstrating short-term improvement in inner retinal function in patients with glaucoma. Further study is warranted to elucidate its long-term effects on glaucoma progression.

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Abstract

Purpose: To investigate whether high-dose nicotinamide (NAM, vitamin B3) can lead to short-term functional gains in glaucoma.

Design: Prospective, crossover, double-masked, interventional, randomized clinical trial.

Participants: Participants diagnosed and treated for glaucoma, aged ≥ 18 years.

Methods: Participants were randomized to receive either placebo (PL) or NAM (vitamin B3) intervention after baseline measurements and reviewed every six weeks. An accelerated dosing method was chosen, whereby participants began with a 6-week course of 1.5 grams/day followed by 6-weeks of 3.0 grams/day. After 12-weeks, participants crossed over without washout and received the other intervention for another 12-weeks. Visual function was measured using the electroretinogram (ERG), including the photopic negative response (PhNR), which reflects retinal ganglion cell activity, and visual field (VF) examination.

Main outcome measures: Changes to retinal function at 12-weeks in placebo and NAM groups as measured using the ERG and VF.

Results: Of the 57 participants randomised, 49 (86%) completed the study. At 12-weeks, NAM-treated participants demonstrated a significant improvement in the PhNR compared to PL group (Vmax, NAM-PL: 1.35 μV [0.159, 2.551], mean [95% confidence interval], p = 0.027; Vmax ratio, NAM-PL: 0.01 [0.002, 0.025], p = 0.023) in the absence of change to intraocular pressure (IOP, p = 0.593). VF mean deviation (MD) demonstrated some per-individual changes but was not overall significant across the group (NAM-PL: 0.10 dB [-0.328, 0.533], p = 0.633). The most frequently reported side effect with NAM was gastrointestinal issues (10.5%).

Conclusions: This world-first clinical trial demonstrates potential for short-term functional improvement in glaucoma with NAM supplementation and warrants further investigation in a larger clinical trial to elucidate its long-term effects on glaucoma progression.

Whilst glaucoma treatment is aimed at lowering intraocular pressure (IOP), methods to protect and potentially recover function in the remaining retinal ganglion cells (RGCs) are lacking. There is increasing evidence that oxidative stress and mitochondrial dysfunction, the principal generators of adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS), play an important role in ageing[1] and the development of glaucoma.[2, 3] Nicotinamide adenine dinucleotide (NAD^+) is an essential cofactor for ATP generation in mitochondria and NAD-dependent enzymes including sirtuins (Figure 1), which play important roles in ageing, cell senescence and stress resistance.[4] Recently, the therapeutic potential of modulating NAD^+ metabolism has gained widespread attention in its potential role in ageing and neurodegenerative disease.[5-9] A finding by Williams et al,[10] demonstrated that NAD^+ supplementation with high-dose nicotinamide (NAM, colloquially known as vitamin B3) or gene therapy with a NAD-producing enzyme (*NMNAT1*, Figure 1) provided strong neuroprotection and reversed age-related changes in an inherited mouse model of glaucoma (DBA/2J). Importantly, NAM supplementation prevented the loss of RGC function, increased RGC survival, and prevented age-related transcriptional and structural changes in mitochondria when delivered as a prophylactic or intervention. Inner retinal function as measured using the pattern electroretinogram (PERG) improved by > 50% in treated mice compared to controls. Recently, it has been shown that people with open-angle glaucoma (OAG) have reduced plasma NAM levels compared to controls, suggesting that NAM supplementation has the potential to play a therapeutic role.[11]

Vitamin B3 encapsulates multiple compounds including niacin/nicotinic acid, which have known ocular and systemic side effects when taken at high doses.[12, 13] In Australia, NAM is a commercially available vitamin B3 supplement and has been previously used in a number of clinical studies with minimal adverse effects.[14] As such, if the therapeutic potential of NAM in glaucoma is realized, the vitamin can be rapidly translated into clinical care to complement current therapies.

We have previously shown that improvement in RGC function, as measured by the photopic negative response (PhNR) of the full-field electroretinogram (ERG), can be detected in as short as 3-months following IOP lowering in glaucoma.[15] Our recent work has also significantly improved the repeatability of the PhNR.[16, 17] This now permits detection of more subtle changes to inner retinal function in response to treatment. Therefore, we sought to determine whether improvements observed in RGC function using high-dose

NAM in the mouse could be replicated in the short-term in clinical glaucoma using the ERG and visual fields (VF).

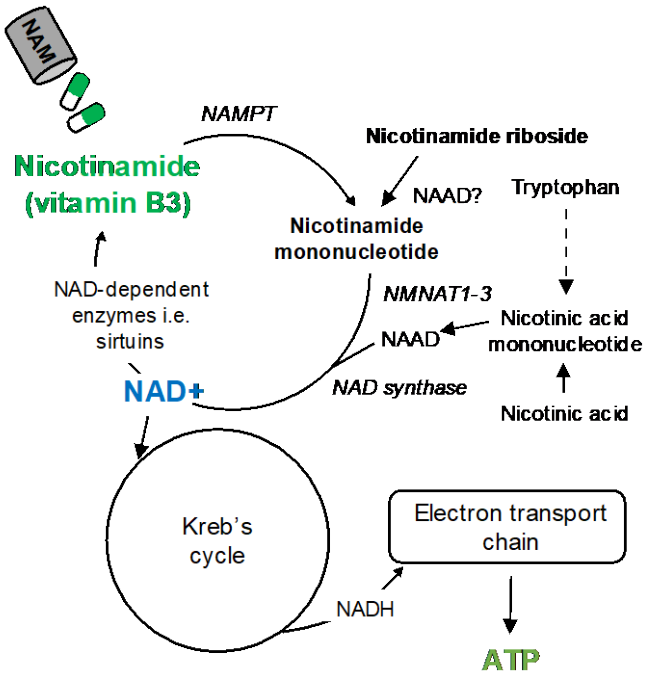


Figure 1. The role of NAD⁺ in ATP production, the pathways through which NAD⁺ is created and salvaged, the major enzymes involved and the process in which NAD⁺ supplements, nicotinamide (green) and nicotinamide riboside, may replete NAD⁺ levels (blue). ATP – adenosine triphosphate, NAAD – nicotinic acid adenine dinucleotide, NAD – nicotinamide adenine dinucleotide, NAM – nicotinamide.

Methods

Study Design and Participants

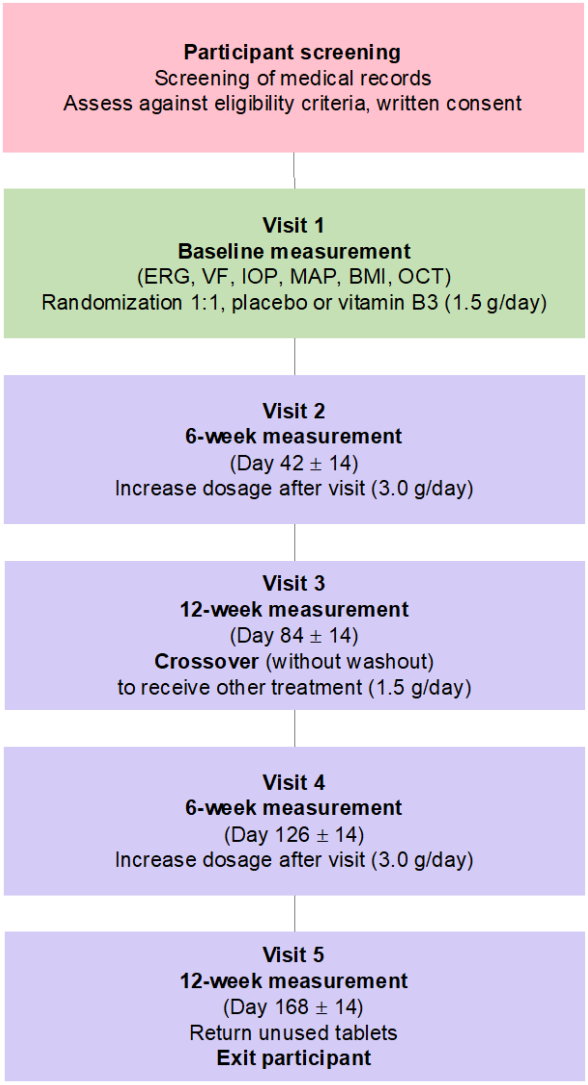
This study was a prospective, double-masked, randomized, crossover clinical trial conducted in Melbourne, Australia between October 2017 – January 2019 (Figure 2). The study was registered on the Australian New Zealand Clinical Trials Registry (ANZCTR, ACTRN12617000809336). At the time of study commencement, there were no registered clinical studies on NAM and visual function. As such, the dosage was based on protocols used in other published[14] and current NAM clinical trials. As this study involved high-dose NAM supplementation, an accelerated dosing protocol was used to facilitate tolerability. Participants were randomized using a randomization schedule generated prior to trial commencement and the order was based on participant enrolment as they were recruited on a rolling basis. Participants were randomized 1:1 to a 6-

week course of either placebo (PL) or vitamin B3 (Insolar®, 0.5 gram NAM per tablet, Blackmores, NSW, Aust) at 1.5 g/day followed by 6-weeks at 3.0 g/day, (1.5 g twice a day, Figure 2). Participants were asked to take the tablets with food. Masked treatment was dispensed at the conclusion of Visit 1 where baseline measurements were taken. Those randomized to receive PL first were labelled as "Treatment Group 1", and those with NAM first as "Treatment Group 2". NAM dosage was based on that used in preclinical studies,[10] which equated to just over 3.0 g/day human dose (based on the weight of a 70 kg adult), and current clinical trials utilising NAM for improving cognitive function in Alzheimer's disease (1.5 g twice a day, ClinicalTrials.gov ID: NCT00580931, NCT03061474). Since study commencement, one new study has been registered, where participants with glaucoma receive 3.0 g NAM with 3.0 g pyruvate orally (NCT03797469).

Participants randomized to receive placebo treatment also doubled the number of tablets after 6-weeks to ensure they remained masked. After 12-weeks, participants crossed over without washout, such that those previously on placebo treatment commenced NAM and vice versa. A washout was deemed unnecessary between the two periods as any effect from NAM was assumed to be undetectable by the time of the next review visit (6-weeks after crossover). Nevertheless, a pre-test for carryover effects was carried out to check this assumption.[18] The treatment was taken in conjunction with any glaucoma therapies participants were already undertaking.

Participants were recruited from outpatient glaucoma clinics (Royal Victorian Eye and Ear Hospital) and a private ophthalmology clinic (Melbourne Eye Specialists). All testing was performed in clinical testing rooms at the Centre for Eye Research Australia. Inclusion criteria included adult participants who were diagnosed and treated for glaucoma, and with visual acuity of at least 6/18. Participants were required to have performed a reliable visual field (SITA-Standard 24-2, Humphrey Field Analyzer, Carl Zeiss Meditec AG, Germany) in the last 6 months, "reliable" being where all reliability indices are < 33%.[19] Where both eyes were eligible, one eye was randomly chosen as the study eye. Exclusion criteria for vitamin B3 supplementation included those who were: pregnant or breast-feeding, unwilling to abstain from other supplements containing vitamin B, allergic to NAM or niacin, diagnosed with cancer in the last 5 years (except treated basal or squamous cell carcinoma), or with a history of liver disease or stomach ulcers. Ophthalmic criteria included: eyes with a history of intraocular surgery in the past 6-months (uncomplicated cataract surgery within the last 3-months) and systemic or ocular

149 diseases that are known to affect retinal function (e.g. age-related macular degeneration, demyelinating
150 diseases, diabetic retinopathy).



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152 *Figure 2. Study design. BMI – body mass index, ERG – electroretinogram, IOP – intraocular pressure, MAP – mean*
153 *arterial pressure, OCT – optical coherence tomography, VF – visual fields.*

154 Each participant was previously diagnosed and treated by a glaucoma specialist, with reproducible VF defects
155 of at least 3 neighbouring points on the total deviation plot with a probability of < 2%.[20] All participants were
156 seen at baseline, and then reviewed 6-weekly (± 2 weeks). At each visit, participants underwent a standard
157 clinical examination, including measurement of visual acuity (Early Treatment Diabetic Retinopathy Study
158 letters converted to logMAR), IOP (Icare® PRO, Icare Finland Oy, Finland), blood pressure (Omron HEM-7322,
159 Omron Healthcare, Japan) and slit lamp examination. Visual fields were performed in one eye (SITA-Standard
160 24-2) and pupils were dilated (to ≥ 6 mm) using 0.5% tropicamide (Bausch and Lomb, NSW, Australia) and 2.5%

phenylephrine (Bausch and Lomb, NSW, Australia). Participants were then light-adapted for at least 10 minutes in the clinical testing room before ERG recording. Photopic ERGs were recorded using custom-made DTL-like electrodes using silver impregnated fibre (22/1 dtex, Shieldex trading, Palmyra, NY, USA) and a handheld device (RETeval^{TM}, LKC Technologies, MD, USA). For optimal PhNR recording, a series of red flashes (621 nm, 16 luminous energies ranging between 0.07 – 12.56 cd.s/m^2, 50 sweeps each, 2 Hz flash interval[21], Figure 3A) on a blue background (470 nm, 10 photopic cd/m^2) was used. Stimuli were calibrated using the ILT-1700 radiometer (International Light Technologies, Newburyport, MA, USA) with a photopic filter in place. Reference and ground gold-cup electrodes (Grass Technologies; Astro-Med Inc. West Warwick, RI, USA) were placed at the temple and forehead respectively. Participants also underwent optical coherence tomography (OCT) to measure retinal nerve fibre layer (RNFL) thickness (Spectralis SD-OCT, Heidelberg Engineering, Dossenheim, Germany). To monitor treatment adherence, participants were asked to bring remaining tablets for counting at each visit. A compliance rate of 70% and above was considered acceptable, which approximately equated to a missed dose twice a week.

Study Objectives

The primary objective was to evaluate the short-term change in ERG and VF after 12 weeks of high-dose NAM supplementation compared to placebo control. Specifically, changes to the PhNR saturating amplitude (PhNR Vmax), PhNR/b-wave ratio (Vmax ratio), VF indices (mean deviation, MD; pattern standard deviation, PSD) were analysed. Secondary objectives included evaluation of alterations to general RNFL thickness, changes to IOP and OPP were also considered. If a significant treatment effect was found at 12-weeks for any parameter, then the 6-week timepoint was also considered.

Ethics Approvals and Consent

All procedures adhered to the tenets of the Declaration of Helsinki and were approved by the Human Research Ethics Committee at the Royal Victorian Eye and Ear Hospital (17/1339H) prior to commencement of the study. Written informed consent was obtained from all participants prior to all procedures.

Sample Size and Power Calculation

Prior to the commencement of the study, sample size was calculated based on the results of a previous pilot study that investigated short-term changes to the PhNR post-IOP lowering (data not shown). To account for

an attrition rate of 20%, it was estimated that 48 eyes were required (power 80%, alpha = 0.05) given an effect size of 0.56, to effectively characterise the functional changes associated with NAD^+ repletion.

ERG Waveform Processing

Custom-written Matlab® scripts (R2018b, Mathworks, MA, USA) were used to process raw ERG traces. ERG waveforms were processed in a masked fashion – the data for each participant was assigned a random number in the code so the assessor could view the data without seeing the timepoint at which it was measured, or the intervention assigned. First, a bandpass filter (0.3 – 300 Hz) was applied. The raw data was detrended with a 3rd order polynomial, which we have previously shown to provide the most robust PhNR signal.[17] From this, the amplitudes and implicit times of the a-wave, b-wave (measured from a-wave trough to b-wave peak) and PhNR (minimum from baseline to trough) were extracted (Figure 3B).

Amplitudes of the b-wave and PhNR across the luminance series were modelled using a saturating hyperbolic function,[22, 23] defined as $V(I) = Vmax*I^n/(I^n + K^n)$, where V (μV) is the amplitude as a function of luminous exposure (I), Vmax is the saturating amplitude (μV), K the semi-saturation constant (1/K is the sensitivity) and n the slope (Figure 3C-D). For fitting the PhNR data, n was fixed to 1.2 (consistent with our pilot data, and similar to other studies).[24, 25] Further, the PhNR Vmax was analysed as a ratio to the b-wave Vmax (Vmax ratio) to account for any potential changes to the b-wave between sessions.

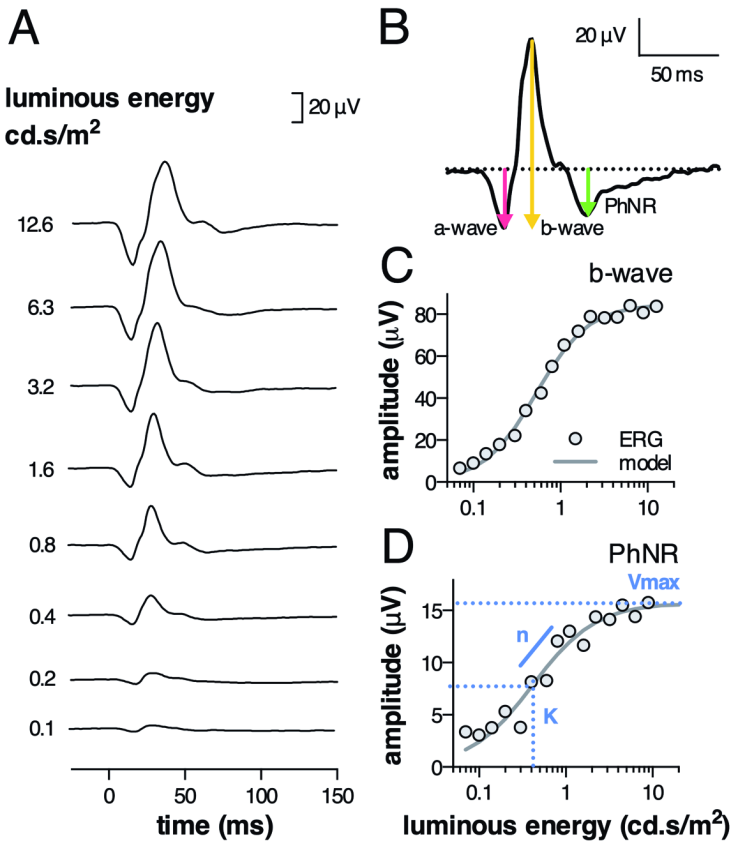

Figure 3. ERG analysis method. A. Representative ERG luminance-response series showing every second luminance step, B. ERG parameters of interest, C. Representative b-wave luminance-response function data (circles) and model derived from a saturating hyperbolic function (line), D. Representative PhNR luminance-response function (circles), corresponding model (line) and model parameters: Vmax (saturating amplitude), n (slope) and K (semi-saturation constant).

Statistical Analysis

The primary analysis set included participants who attended baseline, Visit 3 and Visit 5 (Week 12 visit for each intervention). Statistical analyses were conducted masked to intervention. All analyses were conducted using Stata (SE version 15.1, College Station, Texas, US). The analysis was repeated independently by two independent statisticians using different methodologies but all yielded similar results. One of the analyses is described below.

To test for potential carryover effect, the within-participant sum of values for each parameter of interest was calculated by adding change from baseline values observed under PL to those observed under NAM (at high dose of each). These summed values were compared between randomization groups using two-sample t-tests to investigate the presence of carryover effects.

To test for potential period effect, the within participant difference (NAM-PL) was compared between treatment groups (Treatment group 1 and 2) using linear regression, adjusting for baseline values to assess whether there was any evidence of period on the effect of the treatment.

Due to the crossover study design, the within participant difference (NAM-PL) was derived for each parameter of interest following the high-dose period to examine the treatment effect. Linear regression, with adjustment for mean-centred baseline values, was used to test the null hypothesis of no difference in parameter values between treatments. The intercept term in the regression model was taken as the estimate of treatment difference.

At some visits, the recorded ERG data of some participants could not be used due to excessive muscle twitch and noise artefacts that could not be ameliorated with offline processing. Missing ERG values for these participants were imputed to reduce bias due to missing data and maintain the level of statistical power (detailed in Table 1). Values were multiply imputed (25 imputed datasets) using fully conditional specification (also known as chained equations) with a univariate linear regression imputation model for missing values of ERG parameters. In addition to the imputation variables, imputation models included data on study group, study visit, sex, visual acuity, IOP, ocular perfusion pressure, pupil size, cataract grading, presence of intraocular lens or posterior capsular opacity, and visual field indices (MD, PSD).

As a sensitivity analysis, analyses were repeated using the per-protocol set (n = 43) which only included participants who attended baseline, Visit 3 and Visit 5 and had non-missing ERG data (i.e., a complete case analysis). Results are shown as mean and [95% confidence interval], unless stated otherwise.

Results

Participants

A total of 94 participants were assessed for study eligibility after initial screening of medical records, of whom 57 were randomized into the study. Being unable to commit to 6-weekly reviews was the main reason for participants to decline participating (n = 28, 76%). Of the 57 participants randomized, 49 (86%) completed the study and attended all visits (Figure 4). Of the 8 that did not complete the study, 4 were lost to follow-up at

various timepoints, 2 discontinued the intervention after poor adherence to treatment protocol (< 70% compliance) and another 2 discontinued the intervention where 1 participant developed an adverse event (tinnitus) after commencing placebo treatment and 1 developed a side effect and opted to cease treatment and withdraw from the trial. Data from these 8 participants were not analysed further.

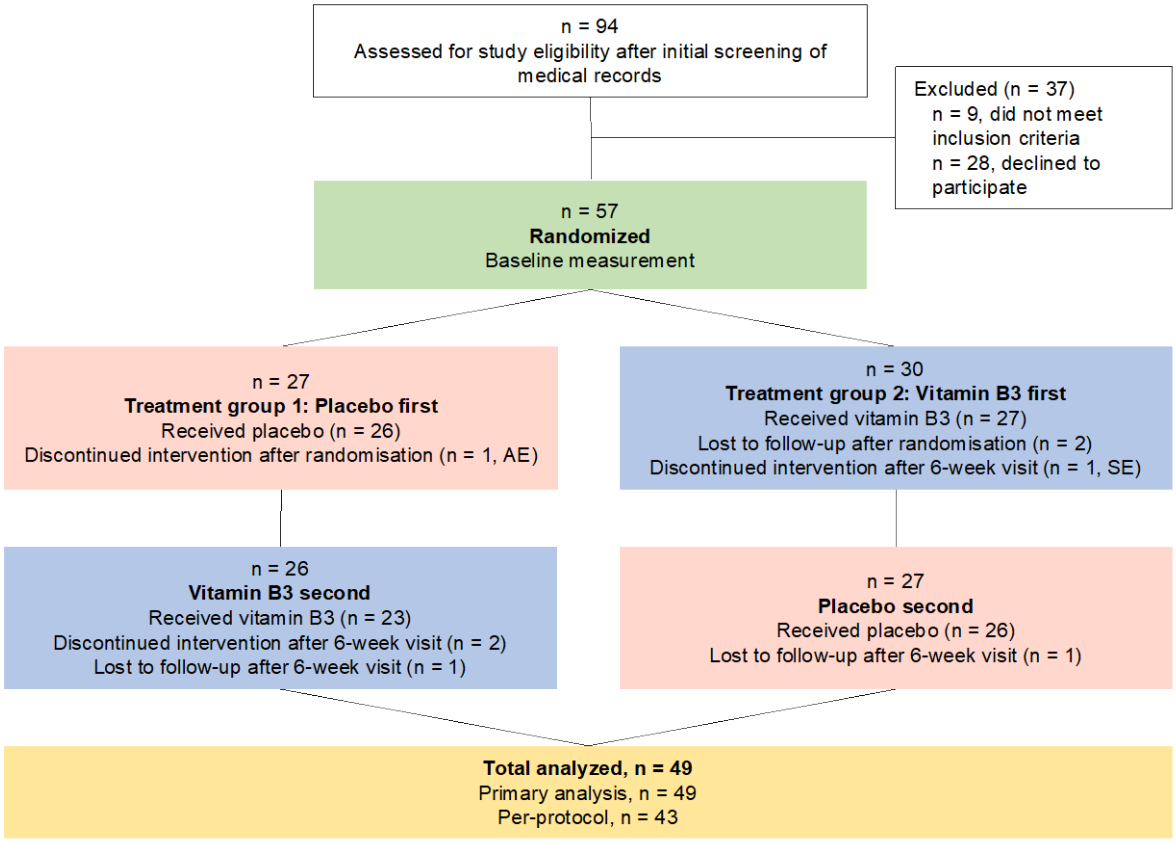

Figure 4. Participants were randomized to receive placebo first (Treatment group 1) or nicotinamide (vitamin B3) first (Treatment group 2). Primary analysis set defined as participants who received both interventions and attended all visits. Per-protocol set defined as a subset of the primary analysis set, excluding any participants with any missing data (n = 6). Participants lost to follow-up (n = 4), participants discontinuing intervention (n = 4). AE – adverse event, SE – side effect.

Participant demographics for the 49 participants included in the primary analysis are shown in Figure 5. The majority of participants had primary open angle glaucoma (POAG, 63%) and median body mass index (BMI) was, males: 26.8 kg/m^2 (interquartile range (IQR): 4.1 kg/m^2), females: 24.1 kg/m^2 (IQR: 5.6 kg/m^2). Participants were randomized to receive PL (Treatment group 1) or NAM (Treatment group 2) first and showed similar baseline characteristics (Table 1). There was one difference, where Treatment Group 1 participants had on

average, a larger PhNR Vmax amplitude at baseline compared to Treatment Group 2 (p = 0.039). In general, all participants were seen within the defined study period (Table 2), with those falling outside the 6 ± 2-week period deemed "lost to follow up" and excluded from the study (n = 4). Compliance rates were high for both interventions and were not statistically different between treatment groups (Table 2). After 12-weeks of NAM supplementation, the compliance rate was 94.4%, [91.5, 96.0] (mean, [Q1, Q3]) compared to PL group with 95.2%, [89.7, 99.2], (p = 0.696) in the first treatment period (Visit 2 & 3). This did not change considerably in the second period (Visit 4 & 5), where compliance rates remained similarly high for both groups (NAM: 96.6%, [93.7, 98.7]; PL: 94.0%, [90.5, 99.2], p = 0.464).

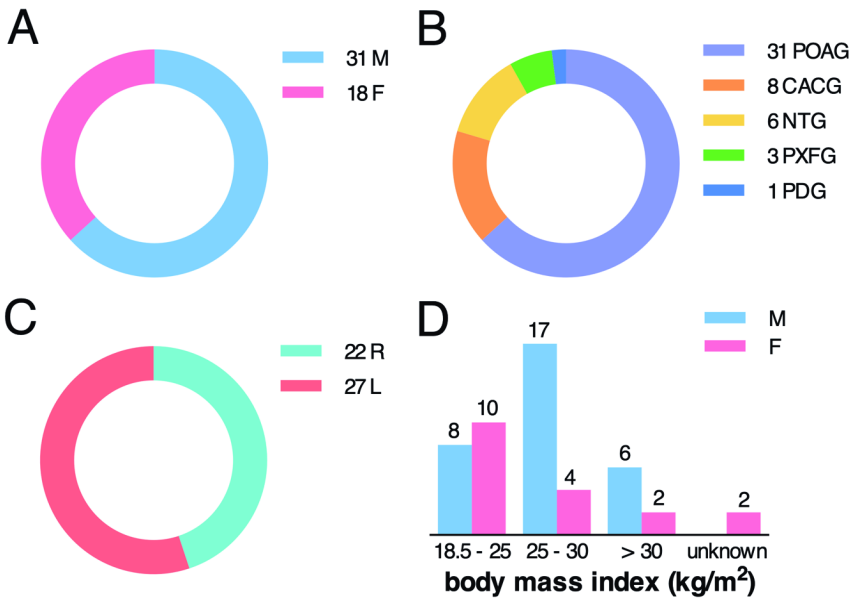

Figure 5. Patient demographics. A. Distribution of sexes (M – male, F – female), B. Glaucoma diagnosis at time of recruitment (POAG – primary open-angle glaucoma, CACG – chronic angle-closure glaucoma, NTG – normal tension glaucoma, PXFG – pseudoexfoliative glaucoma, PDG – pigment dispersion glaucoma,), C. Eye chosen for study (R – right, L – left), D. Distribution of body mass index (BMI, kg/m²) at study commencement between sexes; n = 49.

Table 1. Baseline characteristics for participants in the primary analysis. The two groups are separated by participants that were randomised to receive placebo or nicotinamide first, n = 49 in total. P-values derived from Pearson's χ^2 test (categorical variables), two-sample t-test (normally distributed variables) and Wilcoxon rank-

sum test (skewed continuous variables). ERG – electroretinogram, IOL – intraocular lens, MD – mean deviation, NTG – normal tension glaucoma, POAG – primary open-angle glaucoma, PDG – pigment dispersion glaucoma, PhNR – photopic negative response, PSD – pattern standard deviation, PXFG – pseudoexfoliative glaucoma, Q1 – lower quartile, Q3 – upper quartile, SD – standard deviation, Vmax – saturated amplitude.

	Treatment group 1 Placebo first (n = 23)	**Treatment group 2 Nicotinamide first** (n = 26)	**p-value**
	n (%)	n (%)	
Sex			
Female	10 (43%)	8 (31%)	0.357
Male	13 (57%)	18 (69%)	
Eye tested			
Left	11 (48%)	16 (62%)	0.336
Right	12 (52%)	10 (38%)	
Presence of IOL			
No	12 (52%)	14 (54%)	0.907
Yes	11 (48%)	12 (46%)	
Glaucoma diagnosis			
POAG	14 (61%)	16 (62%)	0.744
CACG	5 (22%)	3 (12%)	
NTG	2 (9%)	5 (19%)	
PXF	1 (4%)	2 (8%)	
PDG	1 (4%)	0 (0%)	
	Mean (SD)	**Mean (SD)**	**p-value**
Age (years)	67.01 (2.05)	65.30 (2.14)	0.569
Visual acuity (logMAR)	0.01 (0.14)	-0.01 (0.11)	0.672
Intraocular pressure (mmHg)	13.6 (2.7)	14.5 (3.3)	0.289
ERG b-wave fitting parameter: n*	1.32 (0.16)	1.29 (0.17)	0.665
ERG b-wave Vmax (μV)*	108.87 (27.48)	99.00 (29.75)	0.261
ERG b-wave fitting parameter: 1/K*	1.22 (0.30)	1.12 (0.41)	0.387
	Median (Q1, Q3)	**Median (Q1, Q3)**	**p-value**
Mean arterial pressure (mmHg)*	92.17 (85.00, 96.00)	95.67 (88.17, 98.67)	0.247
ERG PhNR fitting parameter: 1/K*	2.40 (1.24, 3.64)	1.64 (1.27, 2.68)	0.626
ERG PhNR Vmax (μV)*	15.30 (11.10, 20.58)	11.39 (9.31, 13.49)	0.039
PhNR Vmax/b-wave Vmax ratio*	0.14 (0.11, 0.20)	0.12 (0.10, 0.14)	0.144
Visual field MD (24-2) dB	-5.46 (-7.20, -1.10)	-4.51 (-8.37, -1.84)	0.589
Visual field PSD (24-2) dB	6.77 (2.37, 9.85)	5.89 (3.06, 9.28)	>0.999

* Missing values in Groups 1 and 2, respectively: ERG b-wave fitting parameter: n, maximum amplitude and fitting parameter: 1/K (n = 2 and 3), mean arterial pressure (n = 5 and 2), ERG PhNR (μV), fitting parameter: 1/K (n = 3 and 3), PhNR Vmax/b-wave Vmax ratio (n = 3 and 3).

Table 2. Distribution of days from baseline to follow-up for participants included in the primary analysis and compliance rates (%) at each visit where Treatment group 1 received placebo first, Treatment group received

*nicotinamide first. *p-values derived from Wilcoxon rank-sum test to test for differences in compliance between groups, n = 49 in total. PL – placebo, NAM – nicotinamide, SD – standard deviation.*

Visit no.	Treatment group	Days from baseline			Compliance (%)		
		Target	Mean	(SD)	Median	[Q1, Q3]	p-value*
2 (6 weeks)	Treatment group 1, PL	42	42.5	-6.2	97.6	[88.1, 100.0]	
	Treatment group 2, NAM	42	42.2	-5.0	97.6	[90.5, 100.0]	0.804
3 (12 weeks)	Treatment group 1, PL	84	85.6	-5.0	95.2	[89.7, 99.2]	
	Treatment group 2, NAM	84	85.4	-5.3	94.4	[91.5, 96.0]	0.696
4 (18 weeks)	Treatment group 1, NAM	126	126.8	-5.7	100.0	[97.6, 100.0]	
	Treatment group 2, PL	126	130.4	-8.7	100.0	[92.9, 100.0]	0.885
5 (24 weeks)	Treatment group 1, NAM	168	171.0	-6.3	96.6	[93.7, 98.7]	
	Treatment group 2, PL	168	169.9	-10.5	94.0	[90.5, 99.2]	0.464

Functional Outcomes After 12-weeks of Nicotinamide and Placebo Treatment

Twelve weeks following treatment with placebo or NAM, there were no significant alterations to IOP, MAP or VA in the primary analysis (Table 3; Figure S1 and full statistical output in Table S1 available at www.aaojournal.org). On average, IOP was 13.8 ± 4.1 mmHg in the NAM group compared to 13.4 ± 2.4 mmHg in PL group (NAM-PL: 0.2 mmHg [-0.58, 1.003], p = 0.593).

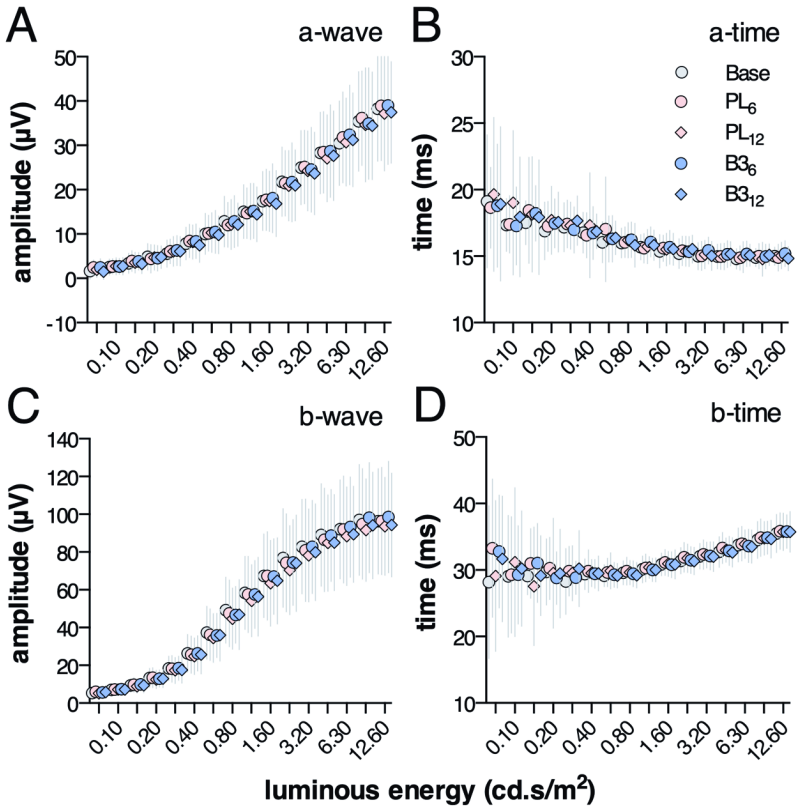

Figure 6. Luminance-response series for the a-wave and b-wave. A-B. No significant change to a-wave amplitude and implicit time following placebo (pink) and vitamin B3 treatment (blue) at 6-weeks (circles) or 12-weeks (diamonds). C-D. No significant change to b-wave amplitudes and implicit times following treatment; n = 43, mean ± SD. PL_6 – placebo at 6-weeks, PL_{12} – placebo at 12-weeks, $B3_6$ – nicotinamide at 6-weeks, $B3_{12}$ – nicotinamide at 12-weeks.

ERG amplitudes and implicit times across the luminance-response series for the a-wave and b-wave did not change significantly (Figure 6). There were no changes to any of the b-wave fitting parameters (Vmax, n, 1/K) between treatment and control groups at both 6 and 12-weeks (Table 3, Figure S2 available at www.aaojournal.org). However, significant improvement was found in the PhNR. Mean difference between treatment and control at 6 & 12-weeks is shown in Figure 7. Both the Vmax ratio (NAM-PL: 0.01 [0.002, 0.025], p = 0.023) and PhNR Vmax (NAM-PL: 1.35 μV [0.159, 2.551], p = 0.027) demonstrated a significant treatment effect at 12-weeks (Figure 7A-B). PhNR sensitivity (1/K) did not change with treatment (p = 0.414, Figure 7C). When comparing the response at 12-weeks post-NAM to baseline values, there was significant group improvement in Vmax ratio (12.6% [5.0, 20.2], p = 0.002, one-sample t-test, Figure 7D, blue diamonds) and PhNR Vmax (14.8% [2.8, 26.9], p = 0.02, Figure 7E). There was no significant carryover effect in any tested parameter (Table S1-S2, available at www.aaojournal.org). Per-protocol analysis results were largely similar to primary analysis, showing significant improvement in the PhNR Vmax and Vmax ratio (Table S2, available at www.aaojournal.org). No significant changes were found at 6-weeks at the lower dosage between NAM and PL groups (Table S3, available at www.aaojournal.org).

Table 3. Primary analysis of the treatment effect. CL – confidence limits, ERG – electroretinogram, IOP – intraocular pressure, MAP – mean arterial pressure, MD – mean deviation, NAM – nicotinamide, PL – placebo, PSD – pattern standard deviation, PhNR – photopic negative response, VA – visual acuity, Vmax – saturating amplitude. P-values estimated via linear regression of NAM-PL difference with adjustment for mean-centred baseline values, n = 49.

	Placebo		Nicotinamide		Baseline-adjusted treatment effect			
	Mean	(SD)	Mean	(SD)	NAM-PL	95% CL		p-value
						Lower	Upper	
VA (logMAR)	-0.03	0.13	-0.03	0.13	0.00	-0.027	0.021	0.825
IOP (mmHg)	13.4	2.4	13.8	4.1	0.20	-0.58	1.003	0.593
MAP (mmHg)	96.55	11.27	95.27	9.73	-1.84	-4.922	1.247	0.235

VF parameters								
MD (dB)	-5.14	4.66	-5.04	4.49	0.10	-0.328	0.533	0.633
PSD (dB)	6.28	3.6	6.04	3.57	-0.25	-0.627	0.136	0.202
ERG parameters								
b-wave Vmax (μV)	102.01	29.07	102.33	30.85	1.11	-5.405	7.626	0.732
b-wave: n	1.25	0.19	1.26	0.22	0.01	-0.057	0.07	0.840
b-wave: 1/K	1.09	0.39	1.08	0.44	-0.01	-0.083	0.067	0.833
PhNR Vmax (μV)	13.48	5.43	14.6	6.08	1.35	0.159	2.551	**0.027**
Vmax ratio	0.14	0.06	0.15	0.07	0.01	0.002	0.025	**0.023**
PhNR: 1/K	2.19	1.71	2.15	1.42	-0.20	-0.683	0.287	0.414

Of the participants that showed an improvement in the PhNR with NAM treatment, 23% (PhNR Vmax, n = 10) and 21% (Vmax ratio, n = 9, Figure 7F, blue) demonstrated a change greater than the coefficient of repeatability (COR, ± 27%) established from our previous work. A smaller proportion of participants on placebo treatment showed a similar change (PhNR Vmax: n = 4, 9%; Vmax ratio: n = 6, 14%, Figure 7F, pink).

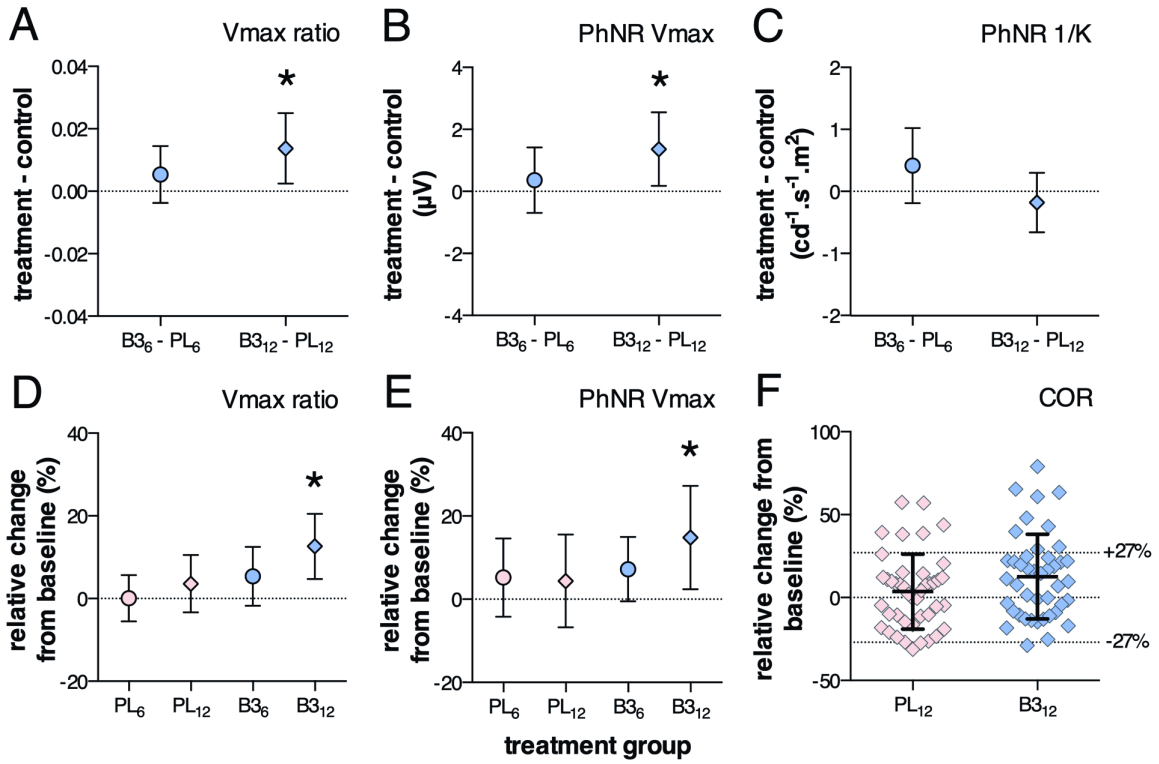

Figure 7. Significant improvement in the PhNR at 12-weeks post NAM intervention. A. Difference in Vmax ratio between nicotinamide (B3) and control (PL) at 6-weeks ($B3_6 – PL_6$, circles) and 12-weeks ($B3_{12} – PL_{12}$, diamonds) showing significant increase at $B3_{12}$ (p = 0.023), B. PhNR Vmax (μV) showing a significant increase at $B3_{12}$ (p = 0.027) , C. PhNR 1/K (sensitivity, $cd^{-1}.s^{-1}.m^2$). D. Vmax ratio expressed as relative change from baseline (%) for

nicotinamide (blue) and control (pink) at 6 and 12-weeks, with a significant change at $B3_{12}$ (p = 0.002, one sample t-test), E. PhNR Vmax ($B3_{12}$, p = 0.02); mean ± 95% CI, n = 43. F. Individual change in Vmax ratio from baseline at 12-weeks in placebo (pink) and nicotinamide (blue) groups, coefficient of repeatability (COR) of ± 27% from our previous work; mean ± SD, n = 43. PL_6 – placebo at 6-weeks, PL_{12} – placebo at 12-weeks, $B3_6$ – nicotinamide at 6-weeks, $B3_{12}$ – nicotinamide at 12-weeks.

Visual Field Indices

In general, there were no significant changes to global VF indices (Table 3). Differences in MD and PSD between NAM and PL groups for each individual is shown in Figure 8. After 12 weeks of NAM supplementation, average MD showed minimal improvement compared to placebo control (NAM-PL: 0.10 dB [-0.328, 0.533], p = 0.633). Similarly, PSD reduced slightly in the NAM group but there was no significant group difference from the PL group (NAM-PL: -0.25 dB [-0.627, 0.136], p = 0.202). There was also no evidence that VF MD and PSD changed on average compared to baseline (Figure 8B & E).

However, we observed a trend for VF MD to improve in the NAM group compared to PL group at 12-weeks. Figure 8C shows that whilst the PL group showed a similar distribution between those that improved (16%) or worsened (12%) by ≥ 1 dB, the NAM group demonstrated a skew towards improvement in VF MD (27% vs. 4%). This was not evident in the PSD, where both NAM and PL groups were similar (Figure 8F).

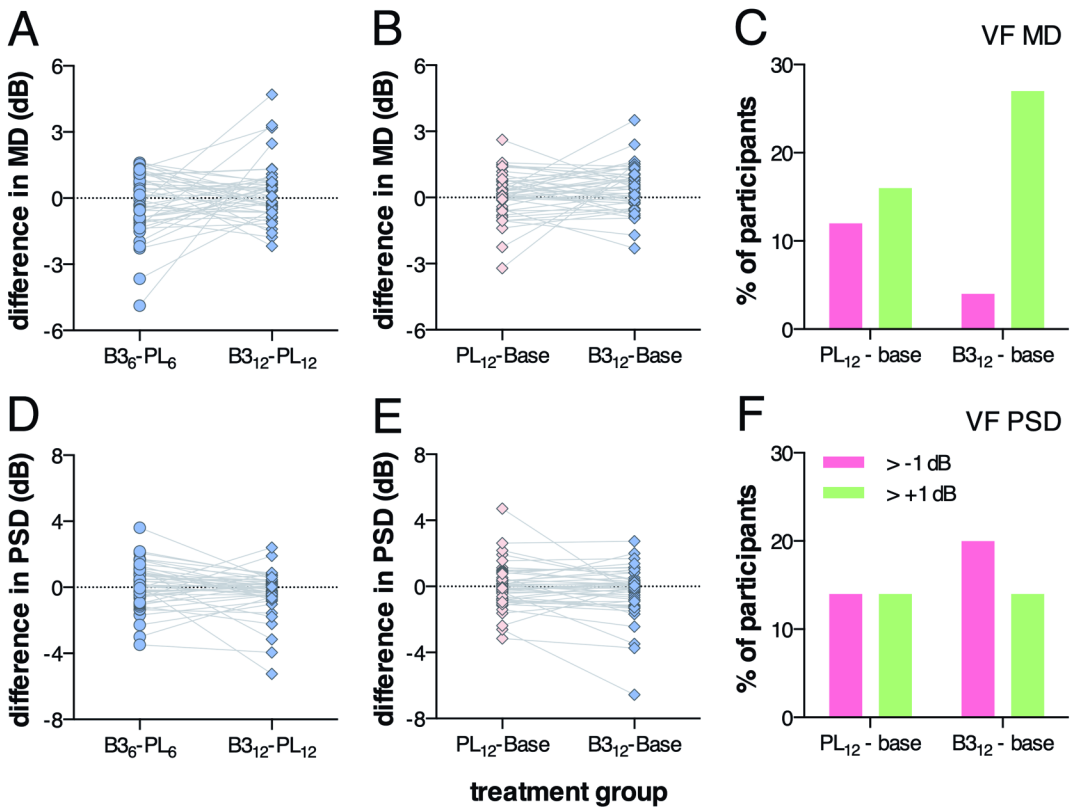

Figure 8. 'Before and after' plots of changes in VF parameters for each individual. A. Difference in VF MD (dB) between treatment (B3) and control (PL) groups at 6 and 12- weeks, B. Difference in MD between PL and B3 groups compared to baseline and 12-weeks, C. Proportion of participants (%) who demonstrated an improvement (green) or worsening (red) of visual field mean deviation (MD) by ≥1 dB compared to baseline, D. Difference in VF PSD (dB) between B3 and PL groups at 6 and 12- weeks, E. Difference in PSD between PL and B3 groups compared to baseline at 12-weeks, F. Proportion of participants (%) who demonstrated an improvement (green) or worsening (red) of visual field pattern standard deviation (PSD) by ≥1 dB compared to baseline; n = 49. Base – baseline, PL_6 – placebo at 6-weeks, PL_{12} – placebo at 12-weeks, $B3_6$ – nicotinamide at 6-weeks, $B3_{12}$ – nicotinamide at 12-weeks.

OCT RNFL at 12-weeks

Overall, there was no significant change to RNFL thickness during the course of the study. At 12-weeks, the difference in RNFL thickness compared to baseline was, NAM: -0.3 ± 2.9 µm compared to PL: 0.4 ± 2.4 µm (mean ± SD, p = 0.109).

Safety and Tolerability

In general, high-dose NAM was quite well tolerated. During the course of the study, two participants withdrew from the study, one of whom experienced a known side effect (gastrointestinal upset) and opted to withdraw.

The most common side effect in the NAM group was gastrointestinal issues (constipation or soft stools; 10.5%) followed by nausea (5.3%) and headaches (3.5%). In the PL group, 12.3% of participants reported difficulty in swallowing the tablets, followed by gastrointestinal upset (7.0%). One participant withdrew after experiencing tinnitus whilst on placebo treatment. All symptoms resolved after participants ceased the intervention.

Discussion

To our knowledge, this is the first clinical investigation of high-dose nicotinamide supplementation in glaucoma. We provide evidence that NAM can generate short-term functional gains in the ERG at 12-weeks, in the absence of IOP lowering. This finding is interesting in light of recent evidence that plasma NAM levels are reduced in people with OAG.[11] Though the group difference was variable as functional improvement was not observed in everyone, this study highlights the need for further study to elucidate whether short-term improvement in inner retinal function with NAM is associated with a reduction in long-term glaucoma progression.

NAD^+ depletion has been associated with ageing[26] and more recently in OAG.[11] The mechanisms by which NAD may improve RGC function are not known. In previous work, we have demonstrated a systemic Complex-I deficiency, where NADH is oxidized into NAD^+. in a subset of POAG eyes.[2, 27] Detailed analysis of OXPHOS complex activity in peripheral blood is a substantial undertaking and was not possible in this cohort. The preclinical work by Williams and colleagues suggest that high-dose NAM may be beneficial in a mouse model of glaucoma in preventing RGC soma loss, RNFL thinning and preserved retinal function as measured by PERG.[10, 28] Significant PERG improvement was seen at both dosages tested and was protective at the prophylactic and interventional level. A similar level of protection was afforded via gene therapy, where the overexpression of *Nmnat1,* a terminal enzyme in NAD^+ synthesis, which prevented RGC axon and soma loss and preserved the PERG. A synergistic effect was found with gene therapy and NAM supplementation. Currently, there are a host of clinical trials on NAD^+ repletion utilising NAD^+ precursors, namely NAM and nicotinamide riboside for a range of conditions, from improving cognition in early Alzheimer's disease (NCT00580931, NCT03061474) to preventing peripheral small fiber neuropathy and promoting nerve regeneration (NCT03912220).

There is growing clinical evidence to suggest that in glaucoma, retinal function can be modulated in the short-term following IOP lowering[15, 29-34] or with bioenergetic substances such as glucose.[35, 36] Casson and colleagues have previously shown that topical 50% glucose drops temporarily improved contrast sensitivity, independent of IOP, in pseudophakic individuals with POAG.[35] There have been many reports of short-term contrast sensitivity and VF improvement with IOP lowering,[29, 33, 34, 37, 38] and some studies have suggested that there may be long-term functional benefits in some patients.[39, 40] Indeed, Gandolfi et al showed that significant improvements in contrast sensitivity observed at 3-9 months post-trabeculectomy persisted for 3 years post-operatively.[29] Caprioli and colleagues also demonstrated on a point-wise VF analysis, long-term improvement in 44% of VF locations five years after trabeculectomy.[39] Due to the short-term nature of the study (5 study visits total over 6-months), we could not perform a reliable point-by-point analysis on the VF results. VF MD and PSD did not change on average (Table 3). However, we observed a trend for VF MD to improve in the NAM group compared to PL group at 12-weeks. This was only observed in the MD and not PSD, which is to be expected as whilst NAM may influence sensitivity thresholds and the hill of vision, the pattern of VF loss should still be evident. Whether this can translate to long-term changes is yet to be determined and is an aim for a future, longitudinal study where additional timepoints can be added to allow for more sophisticated VF analyses.

There has also been evidence of ERG improvement following glaucoma treatment. Improvements in PERG have been reported 3-months after trabeculectomy[30] and following oral acetazolamide.[31] Niyadurupola et al demonstrated a mean 51.8% and 40.2% increase in PhNR amplitude for 2 stimulus intensities (2.25 and 3.00 cd.s/m^2 respectively) following a > 25% IOP reduction.[15] In this study, we found, on average, a 14.8% [2.8, 26.9] improvement in PhNR Vmax amplitude and a 12.6% [5.0, 20.2] improvement in Vmax ratio. Whilst we do not see the same level of improvement in PhNR as Niyadurupola et al, the functional gain we find is independent of IOP lowering. This raises the question whether eyes undergoing a treatment change in glaucoma will have additional benefits with NAM supplementation.

The dosage used in this study was derived empirically from prior preclinical work and available NAM clinical trials at the time of study design (NCT00580931, NCT03061474). One potential caveat is that this dose may not have been sufficient for everyone, as pharmacokinetics and pharmacodynamics will differ between groups, for

example due to age, sex or weight.[41, 42] Here, we did not see a significant correlation between treatment effect and age, sex, or BMI (Figure S3, available at www.aaojournal.org). As recent work suggests that people with OAG have reduced plasma levels of NAM,[11] it would be useful to measure NAM levels following supplementation to elucidate differences between individuals and correlate with treatment outcome measures to explore the underlying mechanisms for improvement. In addition, NAM is relatively safe, it has minimal side effects and is widely commercially available. Future longitudinal studies will reveal the tolerance to long-term supplementation of high-dose NAM. In this study, participants tolerated the NAM supplement quite well, with the most common side effect being gastrointestinal upset (10.5%). However, at the higher dose of 3.0 g/day, participants were required to take 6 tablets per day, (commercial NAM supplements in Australia are only available in 0.5 gram tablets), which led to some occasional adherence issues. Nevertheless, compliance was high for both NAM and PL interventions, maintaining on average, above 90% compliance in both groups.

Here, we enrolled participants who were already treated for glaucoma, thus their IOP tended to be controlled and NAM supplementation demonstrated no further effect on IOP (Figure S1, available at www.aaojournal.org). This removed the potential confounder of improved retinal function due to IOP lowering, as patients presenting with high IOP will usually undergo a change in treatment. This may mean that RGCs in these eyes may be under less stress compared to those in high IOP conditions. Despite the controlled IOP, the underlying disease process remains; however, eyes with high IOP may potentially benefit more from NAM supplementation and can be targeted for recruitment in future studies.

In conclusion, our study of demonstrates short-term improvement in inner retinal function in glaucoma patients in response to NAM supplementation. A larger, longitudinal clinical trial is warranted to explore whether these short-term functional gains of NAM will affect long-term glaucoma progression.

Supplementary material

This article contains additional online-only material. The following should appear online-only: Figure S1 – S3, and Tables S1 – S3.

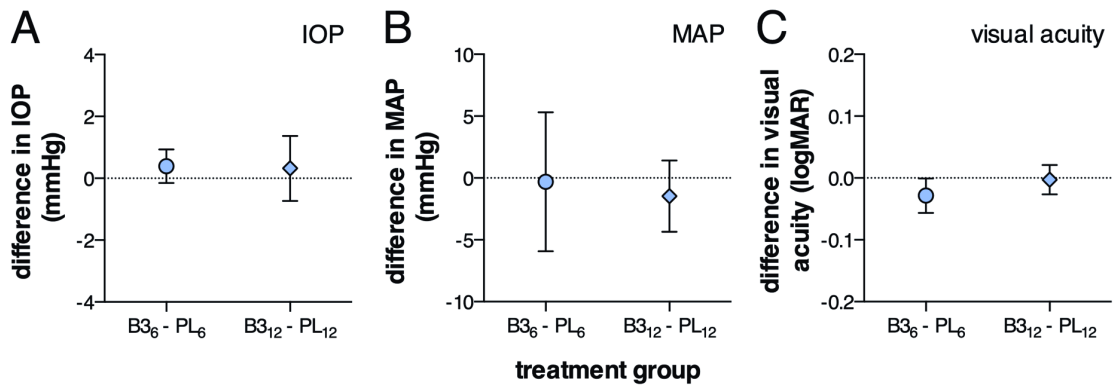

Figure S1. No change in intraocular pressure (IOP), mean arterial pressure (MAP) and visual acuity. A. Difference in IOP between treatment and control groups at 6-weeks (circles) and 12-weeks (diamonds, p = 0.593), B. Difference in MAP (p = 0.235), C. Difference in visual acuity (logMAR scale, p = 0.825); mean ± 95% CI, linear regression with adjustment for mean-centred baseline values, n = 49. PL$_6$ – placebo at 6-weeks, PL$_{12}$ – placebo at 12-weeks, B3$_6$ – nicotinamide at 6-weeks, B3$_{12}$ – nicotinamide at 12-weeks.

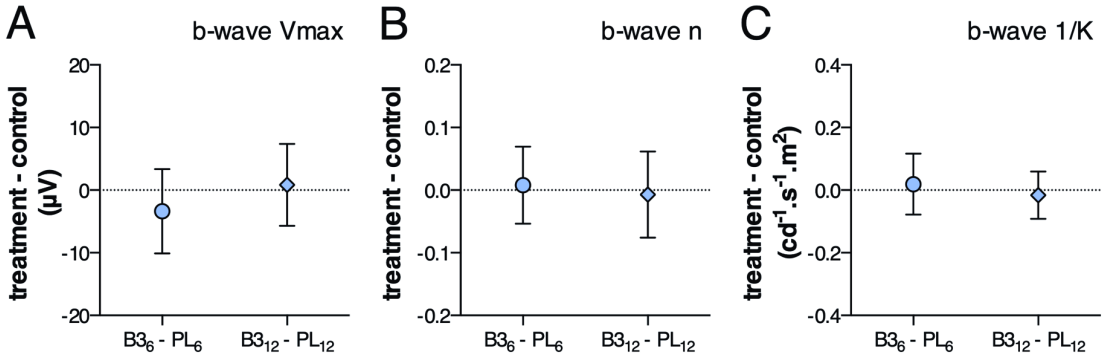

Figure S2. No difference in b-wave fitting parameters between treatment and control groups. A. Difference in b-wave Vmax between treatment (B3) and control (PL) at 6-weeks (circles) and 12-weeks (diamonds, p = 0.732), B. b-wave n (p = 0.840), C. b-wave 1/K (p = 0.833); mean ± 95% CI, linear regression with adjustment for mean-centred baseline values, n = 43. PL$_6$ – placebo at 6-weeks, PL$_{12}$ – placebo at 12-weeks, B3$_6$ – nicotinamide at 6-weeks, B3$_{12}$ – nicotinamide at 12-weeks.

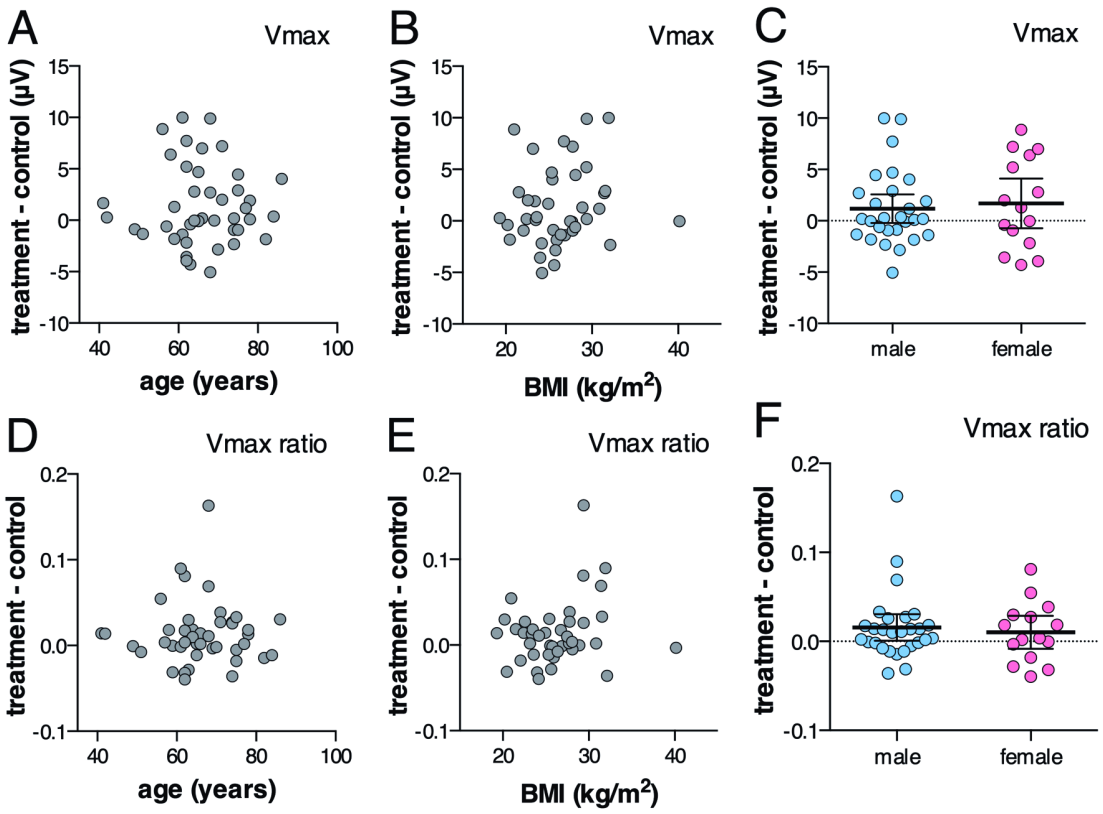

Figure S3. No correlation between treatment effect in the ERG and demographic parameters. A-C. Change in PhNR Vmax (treatment – control) versus age (years), BMI (kg/m²) and sex (p = 0.68, unpaired t-test, mean ± 95% CI), D-F. Change in Vmax ratio (treatment – control) versus age (years), BMI (kg/m²) and sex (p = 0.65). Vmax – saturated amplitude, n = 43.

Table S1. Primary analysis of treatment effect at 12-weeks. P-values estimated via linear regression of NAM-PL difference with adjustment for mean-centred baseline values, n = 49. ERG – electroretinogram, NAM – nicotinamide, PhNR – photopic negative response, PL – placebo, SD – standard deviation.

	Carryover effect	Period effect	Placebo		Nicotinamide		Baseline-adjusted treatment effect			
								95% confidence limits		
	p-value	p-value	Mean	(SD)	Mean	(SD)	NAM-PL	Lower	Upper	p-value
Visual acuity (logMAR)	0.86	0.155	-0.03	0.13	-0.03	0.13	0.00	-0.027	0.021	0.825
Intraocular pressure (mmHg)	0.319	0.378	13.4	2.4	13.8	4.1	0.20	-0.58	1.003	0.593
Mean arterial pressure (mmHg)	0.317	0.148	96.55	11.27	95.27	9.73	-1.84	-4.922	1.247	0.235
Visual field parameters (24-2)										
Mean deviation (dB)	0.468	0.49	-5.14	4.66	-5.04	4.49	0.10	-0.328	0.533	0.633
Pattern standard deviation (dB)	0.087	0.114	6.28	3.6	6.04	3.57	-0.25	-0.627	0.136	0.202
ERG parameters										
b-wave Vmax (μV)	0.199	0.203	102.01	29.07	102.33	30.85	1.11	-5.405	7.626	0.732
b-wave fitting parameter: n	0.301	0.195	1.25	0.19	1.26	0.22	0.01	-0.057	0.07	0.840
b-wave fitting parameter: 1/K	0.795	0.296	1.09	0.39	1.08	0.44	-0.01	-0.083	0.067	0.833
PhNR Vmax (μV)	0.368	0.21	13.48	5.43	14.6	6.08	1.35	0.159	2.551	**0.027**
PhNR fitting parameter: 1/K	0.27	0.034	2.19	1.71	2.15	1.42	-0.20	-0.683	0.287	0.414
Vmax ratio	0.906	0.885	0.14	0.06	0.15	0.07	0.01	0.002	0.025	**0.023**

Table S2. Per-protocol analysis of treatment at 12-weeks. P-values estimated via linear regression of NAM-PL difference with adjustment for mean-centred baseline values, n = 43. ERG – electroretinogram, NAM – nicotinamide, PhNR – photopic negative response, PL – placebo, SD – standard deviation.

	Carryover effect	Period effect	Placebo		Nicotinamide		Baseline adjusted treatment effect			
								95% confidence limits		
	p-value	p-value	Mean	(SD)	Mean	(SD)	NAM-PL	Lower	Upper	p-value†
Visual acuity (logMAR)	0.732	0.139	-0.04	0.12	-0.05	0.10	-0.01	-0.039	0.011	0.268
Intraocular pressure (mmHg)	0.45	0.235	13.5	2.5	13.8	4.2	0.2	-0.702	1.04	0.698
Mean arterial pressure (mmHg)	0.276	0.108	96.59	11.62	95.2	9.47	-2.11	-5.338	1.119	0.193
Visual field parameters (24-2)										
Mean deviation (dB)	0.841	0.672	-4.92	4.51	-4.81	4.43	0.12	-0.358	0.596	0.618
Pattern standard deviation (dB)	0.125	0.205	6.06	3.63	5.75	3.55	-0.33	-0.753	0.093	0.123
ERG parameters										
b-wave Vmax (μV)	0.137	0.187	102.02	26.34	102.84	27.49	1.01	-5.639	7.665	0.76
b-wave fitting parameter: n	0.353	0.146	1.26	0.18	1.27	0.21	0.01	-0.056	0.073	0.793
b-wave fitting parameter: 1/K	0.93	0.405	1.12	0.36	1.1	0.42	-0.01	-0.089	0.062	0.725
PhNR Vmax (μV)	0.222	0.209	13.51	4.7	14.88	5.52	1.35	0.16	2.549	**0.027**
PhNR fitting parameter: 1/K	0.148	0.034	2.27	1.45	2.08	1.2	-0.20	-0.682	0.286	0.413
Vmax ratio	0.857	0.885	0.14	0.05	0.15	0.06	0.01	0.002	0.025	**0.022**

Table S3. Per-protocol analysis of treatment effect at 6-weeks. P-values estimated via linear regression of NAM-PL difference with adjustment for mean-centred baseline values, n = 42. ERG – electroretinogram, NAM – nicotinamide, PhNR – photopic negative response, PL – placebo, SD – standard deviation.

	Carryover effect	Period effect	Difference between baseline & 6-weeks				Baseline-adjusted treatment effect			
			Placebo		Nicotinamide		NAM-PL	95% confidence limits		p-value†
	p-value	p-value	Mean	(SD)	Mean	(SD)		Lower	Upper	
Visual acuity (logMAR)	0.92	0.413	-0.02	0.121	-0.04	0.11	-0.02	0.01	-0.05	0.195
Intraocular pressure (mmHg)	0.349	0.676	13.63	2.778	13.95	2.97	0.28	0.91	-0.36	0.383
Mean arterial pressure (mmHg)										
Visual field parameters (24-2)										
Mean deviation (dB)	0.23	0.298	-4.77	4.682	-4.77	5.08	-0.03	0.35	-0.41	0.876
Pattern standard deviation (dB)	**0.014**	**0.039**	5.71	3.585	5.80	3.71	0.11	0.54	-0.32	0.612
ERG parameters										
b-wave Vmax (μV)	0.145	0.786	107.6	36.251	105.48	29.45	-2.24	5.66	-10.15	0.569
b-wave fitting parameter: n	0.235	0.595	1.26	0.181	1.26	0.20	0.00	0.06	-0.06	0.965
b-wave fitting parameter: 1/K	0.602	0.363	1.09	0.376	1.12	0.40	0.04	0.14	-0.07	0.474
PhNR Vmax (μV)	0.897	0.108	14.08	5.714	14.57	6.35	0.50	1.58	-0.58	0.355
PhNR fitting parameter: 1/K	0.195	0.927	2.30	1.231	2.72	2.09	0.50	1.05	-0.04	0.067
Vmax ratio	0.324	0.432	0.14	0.059	0.14	0.06	0.01	0.01	0.00	0.252

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