1 **Improvement in Inner Retinal Function in Glaucoma with Nicotinamide**

2 **(Vitamin B₃) Supplementation: A Crossover Randomised Clinical Trial**

3 **Authors:** Flora Hui, PhD[1,†], Jessica Tang, MBBS(Hons) PhD[1,2], Pete A Williams, PhD[3],

4 Myra B McGuinness, PhD[1], Xavier Hadoux, PhD[1], Robert J Casson, DPhil FRANZCO[4],

5 Michael Coote, MBBS FRANZCO[1,2], Ian A Trounce PhD[1,2], Keith R. Martin, DM

6 FRCOphth[1,2,5], Peter van Wijngaarden, PhD FRANZCO[1,2], Jonathan G Crowston, PhD

7 FRANZCO[1,2,6,7]

8

9 [1] Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne,

10 Australia

11 [2] Ophthalmology, Department of Surgery, University of Melbourne, Melbourne,

12 Australia

13 [3] Department of Clinical Neuroscience, Division of Eye and Vision, St. Erik Eye

14 Hospital, Karolinska Institutet, Stockholm, Sweden

15 [4] Ophthalmic Research Laboratories, Discipline of Ophthalmology and Visual Sciences,

16 University of Adelaide, Adelaide, Australia

17 [5] Department of Clinical Neurosciences, University of Cambridge, Cambridge, United

18 Kingdom

19 [6] Centre for Vision Research, Duke-NUS Medical School, Singapore

20 [7] Singapore Eye Research Institute, Singapore National Eye Centre, Singapore

21 [†] Corresponding author

26 Eye Research Australia receives operational infrastructure support from the Victorian

27 Government. The funding organisations had no role in the design or conduct of this

28 research.

29 **Running head:** Nicotinamide improves retinal function in glaucoma

30 **Word count:** 3995

31 **Abbreviations and Acronyms:** ATP – adenosine triphosphate, CI – confidence interval,

32 COR – coefficient of repeatability, ERG – electroretinogram, IOL – intraocular lens, IOP

33 – intraocular pressure, IQR – interquartile range, MD – mean deviation, MLS – mean

34 light sensitivity, NAD^+ – nicotinamide adenine dinucleotide, NAM – nicotinamide, PL –

35 placebo, POAG – primary open-angle glaucoma, PhNR – photopic negative response,

36 PSD – pattern standard deviation, Q1 – lower quartile, Q3 – upper quartile, RGC – retinal

37 ganglion cell, RNFL – retinal nerve fibre layer, VF – visual field, Vmax – saturating

38 amplitude

39 **Corresponding author:**

40 Dr Flora Hui

41 Centre for Eye Research Australia

42 Level 7, Peter Howson Wing, 32 Gisborne Street

43 East Melbourne, VIC 3002, Australia

44 f.hui@unimelb.edu.au

45 **Conflict of interest:** No conflicting relationship exists for any author.

46 **Data sharing statement**: Clinical data was collected at the Centre for Eye Research

47 Australia in collaboration with the Royal Victorian Eye and Ear Hospital and Melbourne

48 Eye Specialists. These data are not publicly available. Source data of graphs may be

49 available as source files if requested. A description of the code used to analyse the

50   electroretinogram data is described in the Methods section, and we have also published

51   this previously (Tang et al, 2018).

Abstract

Importance: Retinal ganglion cells endure significant metabolic stress in glaucoma but maintain capacity to recover function. Nicotinamide, a precursor of NAD+, is low in serum of glaucoma patients and its supplementation provides robust protection of retinal ganglion cells in preclinical models. However, the potential of nicotinamide in human glaucoma is unknown.

Background: To examine the effects of nicotinamide on inner retinal function in glaucoma, in participants receiving concurrent glaucoma therapy.

Design: Crossover, double-masked, randomised clinical trial. Participants recruited from two tertiary care centres.

Participants: Fifty-seven participants, diagnosed and treated for glaucoma.

Methods: Participants received oral placebo or nicotinamide and reviewed six-weekly. Participants commenced 6-weeks of 1.5 grams/day then 6 weeks of 3.0 grams/day followed by crossover without washout. Visual function measured using electroretinography and perimetry.

Main outcome measures: Change in inner retinal function, determined by photopic negative response (PhNR) parameters: saturated PhNR amplitude (Vmax), ratio of PhNR/b-wave amplitude (Vmax ratio).

Results: PhNR Vmax improved beyond 95% coefficient of repeatability (COR) in 23% of participants following nicotinamide versus 9% on placebo. Overall, Vmax improved by 14.8% [95% CI: 2.8%, 26.9%], (p=0.02) on nicotinamide and 5.2% [-4.2%, 14.6%], (p=0.27) on placebo. Vmax ratio improved by 12.6% [5.0%, 20.2%], (p=0.002) following nicotinamide, 3.6% [-3.4%, 10.5%], (p=0.30) on placebo. A trend for improved visual field mean deviation was observed with 27% improving ≥1dB on nicotinamide and fewer deteriorating (4%) compared to placebo (p=0.02).

77 **Conclusions:** Nicotinamide supplementation can improve inner retinal function in

78 glaucoma. Further studies underway to elucidate the effects of long-term nicotinamide

79 supplementation.

80 **Trial Registration:** ANZCTR trial ID: ACTRN12617000809336

81 https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=373001

82 **Keywords:** nicotinamide, glaucoma, electroretinography, neuroprotection, vitamin B3

84 **Introduction**

85 Glaucoma remains the leading cause of irreversible blindness worldwide.[1] The disease

86 has a complex aetiology and range of risk factors but is characterised by gradual

87 dysfunction and loss of retinal ganglion cells (RGCs) and their axons which make up the

88 optic nerve.[2] This slow, progressive loss of visual function hinders the translation of

89 potential neuroprotective therapeutics from bench-to-bedside due to the extended time

90 needed to determine treatment-induced changes in glaucoma progression rates. Age,[1]

91 genetics,[3] and elevated intraocular pressure (IOP)[4] are all major risk factors for glaucoma

92 yet clinically available treatment strategies target only IOP-lowering, and do not directly

93 target the neurodegenerative events at the level of the retina and optic nerve. As many

94 patients are refractory to IOP lowering or progress to blindness despite low IOP,

95 neuroprotective strategies that directly target RGC health and decrease vulnerability to

96 glaucoma-related cellular stressors are urgently needed. Accumulating evidence points

97 to the potential for visual recovery in clinical glaucoma in response to IOP-lowering.[5-7]

98 Recovery of visual function can occur soon after restoration of IOP and, as such,

99 objective measures of visual function have potential as surrogate biomarkers of

100 improved RGC health.

101 The photopic negative (PhNR) and positive scotopic threshold responses are

102 parameters of the electroretinogram (ERG) that are largely generated by RGCs in the

103 inner retina.[8-10] RGC function has been shown to recover after acute and chronic IOP

104 insults even after prolonged periods of functional loss.[11-13] Capacity for recovery is

105 reduced with advancing age[14] and can be modified by interventions such as exercise

106 and diet restriction.[15,16] Using ERG protocols similar to those validated in rodent studies,

107 we have demonstrated improvements in RGC function in humans, as early as 3-months

108 following IOP-lowering in glaucoma.[5] Recent work has significantly improved

109   repeatability of the PhNR measurement, enabling detection of subtle changes in inner

110   retinal function.[17,18] Importantly, the PhNR can be easily measured in clinic compared to

111   the scotopic threshold response which requires dark adaptation[19] and the pattern ERG,

112   which requires refractive error correction.[20]

113   Several lines of evidence implicate oxidative stress and mitochondrial dysfunction in

114   ageing[21] and RGC loss in glaucoma.[22,23] Nicotinamide adenine dinucleotide (NAD^+) is an

115   essential cofactor for ATP generation in mitochondria, and for NAD-consuming enzymes

116   including sirtuins (Figure 1), with important roles in ageing, cell senescence, and stress
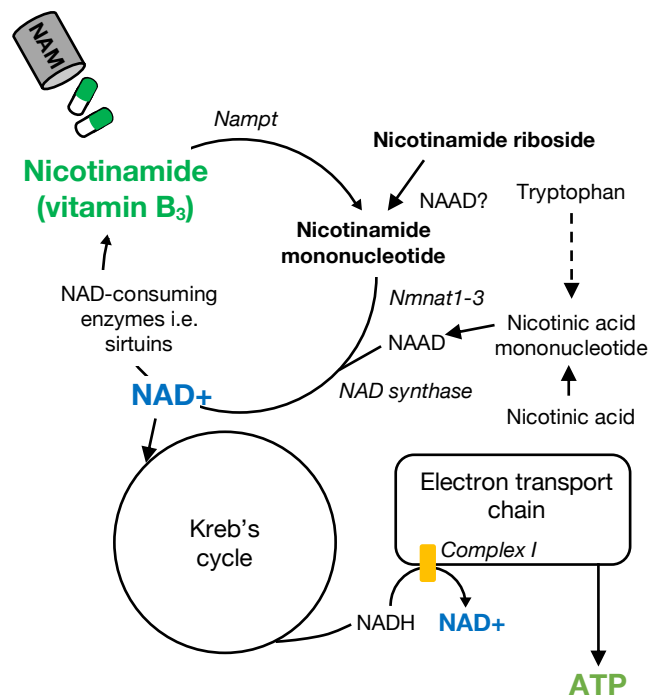
117   resistance.[24]

118

119   **Figure 1. The role of nicotinamide adenine dinucleotide (NAD^+, blue) in adenosine**

120   **triphosphate (ATP) production.** *The pathways through which NAD^+ is created and*

121   *salvaged, the major enzymes involved and the process in which NAD^+ supplements,*

122   *nicotinamide (green) and nicotinamide riboside, may replete NAD^+ levels. Complex I*

123   *(yellow) within the electron transport chain, where nicotinamide adenine dinucleotide +*

124 *hydrogen (NADH) is oxidized to NAD+ and site of potential mitochondrial dysfunction in*

125 *glaucoma. NAAD – nicotinic acid adenine dinucleotide.*

126

127 NAD+ is a potent mediator of axon neuroprotection and recently, the therapeutic potential

128 of modulating NAD+ metabolism has gained widespread attention in its role in ageing

129 and neurodegenerative disease.[25-27] A number of studies are currently investigating the

130 effect of NAD+ repletion in a range of neurodegenerative disorders including Alzheimer's

131 disease (NCT00580931, NCT03061474) and peripheral small fibre neuropathy

132 (NCT03912220). Recent work has indicated that patients with primary open-angle

133 glaucoma (POAG) have reduced serum levels of nicotinamide (NAM, the amide of

134 vitamin B_3 and precursor for NAD+), indicating that systemic NAD+ levels may be

135 associated with susceptibility to glaucoma.[28] In support of this hypothesis, Williams et

136 al[29] demonstrated that increasing retinal NAD+ levels with dietary NAM or overexpression

137 of a NAD-producing enzyme (*Nmnat1*) provided robust long-term neuroprotection of

138 RGCs, reversed age-related transcriptomic changes, and preserved RGC function in an

139 inherited mouse model of glaucoma (DBA/2J). These findings support the hypothesis

140 that NAM supplementation has therapeutic potential. In addition, NAM has been safely

141 used in a number of clinical studies with minimal adverse effects[30,31] and, as such, NAM

142 supplementation may be readily translated into clinical care and complement current

143 therapies.

144 We therefore sought to determine whether functional improvements observed with NAM

145 supplementation in mice could be recapitulated in humans with glaucoma in an

146 interventional study using the ERG PhNR and visual fields (VF) as main outcome

147 measures. This study sought to determine whether NAM, a potential neuroprotectant

148   that targets bioenergetic insufficiency in glaucoma, could lead to a detectable

149   improvement in inner retinal function.

150

151   **Methods**

152   **Study Design, Participants**

153   This study was a prospective, double-masked, randomized, crossover clinical trial

154   conducted in Melbourne, Australia between October 2017 and January 2019. The study

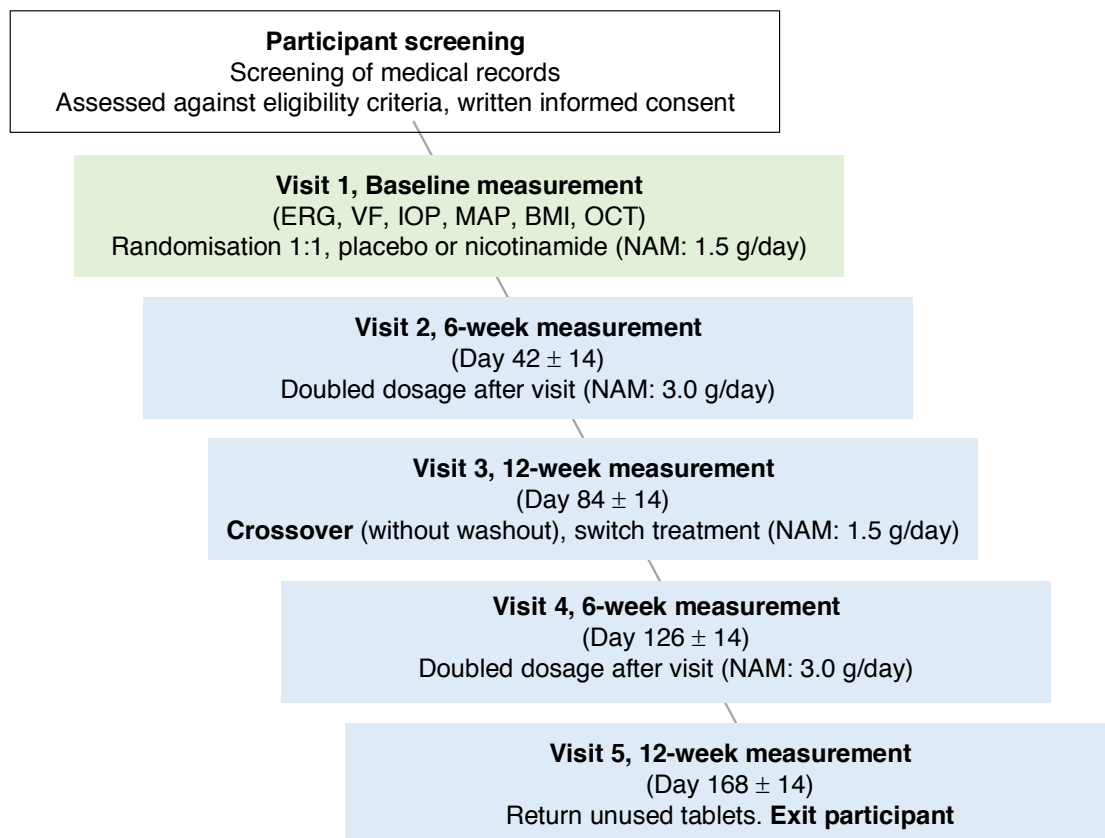155   schedule is presented in Figure 2.

156

157   ***Figure 2. Study schedule.*** *BMI – body mass index, ERG – electroretinogram, IOP –*

158   *intraocular pressure, MAP – mean arterial pressure, NAM – nicotinamide, OCT – optical*

159   *coherence tomography, VF – visual fields.*

160

161 All procedures were approved by the Human Research Ethics Committee at the Royal

162 Victorian Eye and Ear Hospital (17/1339H). Written informed consent was obtained from

163 all participants prior to all procedures. Participants were recruited from public outpatient

164 and private ophthalmology clinics (Royal Victorian Eye and Ear Hospital, Melbourne Eye

165 Specialists). All testing was performed at the Centre for Eye Research Australia.

166 Inclusion criteria included adult participants diagnosed and treated for early-moderate

167 glaucoma (VF mean deviation $\geq$ -10dB) by a sub-specialist ophthalmologist, and with

168 visual acuity of at least 6/18. Participants were required to have performed a reliable VF

169 (SITA-Standard 24-2, HFA II-750i, Carl Zeiss Meditec AG, Germany) in the last 6 months,

170 with <33% fixation losses, false positives and false negatives.[32] Eligible participants had

171 reproducible VF defects of at least 3 neighbouring points with a sensitivity below the age-

172 matched normal (total deviation) with a probability of <2%.[33] Where both eyes were

173 eligible, the eye with the better VF was chosen as we expect eyes with milder disease

174 to be more amenable to treatment.

175 Exclusion criteria included pregnancy/breastfeeding, allergy to NAM/niacin, history of

176 cancer in the last 5 years (except treated basal or squamous cell carcinoma), liver

177 disease or stomach ulcers. Ophthalmic exclusion criteria included intraocular surgery in

178 the past 6 months (uncomplicated cataract surgery within 3 months) and diseases known

179 to affect retinal function (e.g. age-related macular degeneration, diabetic retinopathy).

180

181 As this study involved high-dose NAM, an accelerated dosing protocol was used to

182 facilitate tolerability. Participants were randomised 1:1 with randomly selected block

183 sizes of 4, 6 and 8 using a computer-generated list to the intervention in either the first

184 or second study period. The intervention consisted of a 6-week course of NAM (Insolar®,

185 0.5 g NAM tablet, Blackmores, NSW, Australia) of 1.5 g/day followed by 6 weeks of 3.0

186   g/day, (1.5 g twice a day, morning and evening). Placebo (PL) tablets were manufactured

187   to match the appearance and texture of the active treatment (Pharmaceutical Packaging

188   Professionals, Melbourne, Australia). Placebo and active treatment bottles were identical

189   and coded with unique numerical identifiers. Participants randomised to receive placebo

190   also doubled the number of tablets after 6 weeks to ensure they remained masked.

191   Treatment was dispensed by the study coordinator at the conclusion of Visit 1 following

192   capture of baseline measurements. Participants, treating physicians, certified assessors

193   and biostatisticians were masked to treatment allocation. The NAM dosage of 3.0 g/day

194   was based on that used in preclinical studies,[29] other published[30] and ongoing human

195   trials of NAM treatment (NCT00580931, NCT03061474).

196

197   After 12 weeks, participants crossed over without washout, such that those previously

198   on placebo treatment commenced NAM and vice versa. A washout was deemed

199   unnecessary as any effect from NAM was assumed to be undetectable by the time of

200   the next review visit. Treatment was taken in conjunction with any glaucoma therapies

201   participants were using. To monitor treatment adherence, participants were asked to

202   bring remaining tablets to each study visit and tablets were manually counted. A

203   minimum adherence rate of 70% was acceptable, equating to no more than two missed

204   doses a week.

205   **Clinical Testing**

206   All participants were seen at baseline (Visit 1), and reviewed 6-weekly ($\pm$2 weeks). At

207   each visit, participants underwent a standard clinical examination, including

208   measurement of visual acuity (EDTRS letters), IOP (Icare® PRO, Icare Finland Oy,

209   Finland), blood pressure (HEM-7322, Omron Healthcare, Japan) and slit lamp

210   examination. The use of a rebound tonometer reduced the number of drops administered

211   at each visit. Further, the Icare® PRO has demonstrated repeatability and comparability

212   to Goldmann applanation tonometry.[34,35] Importantly, in our study, IOP measurements

213   were taken using the same device. If large variations in measurements occurred, a

214   repeated measure was taken to confirm the IOP. If a "Deviation" was displayed on the

215   device which indicates a ≥15% deviation amongst the 6 measurements, then the

216   measurement was discarded and repeated. Standard automated perimetry was

217   performed (SITA-Standard 24-2, HFA II-750i, Carl Zeiss Meditec AG, Germany) and

218   pupils were dilated (to ≥6 mm) using 0.5% tropicamide and 2.5% phenylephrine (Bausch

219   and Lomb, NSW, Australia). Participants were light-adapted for at least 10 minutes

220   before photopic ERG recording. ERGs were recorded with custom-made DTL-like

221   electrodes using silver impregnated fibre (22/1 dtex, Shieldex trading, NY, USA) and a

222   handheld device (RETeval™, LKC Technologies, MD, USA). Reference and ground

223   gold-cup electrodes (Grass Technologies, Astro-Med Inc., RI, USA) were placed at the

224   temple and forehead respectively. For optimal PhNR recording, a series of red flashes

225   (621 nm, 16 luminous energies between 0.07-12.56 $cd.s/m^2$, 50 sweeps, 2 Hz flash

226   interval,[36] Figure 3A) on a blue background (470 nm, 10 photopic cd/m^2) was used.

227   Stimuli were calibrated using the ILT-1700 radiometer (International Light Technologies,

228   MA, USA) with a photopic filter. Participants underwent optical coherence tomography

229   (OCT) to measure circumpapillary retinal nerve fibre layer (RNFL) thickness (Spectralis

230   SD-OCT, Heidelberg Engineering, Dossenheim, Germany).

231   **Study Objectives**

232   The primary objective was to evaluate change in retinal function as measured by ERG

233   parameters after 12 weeks of high-dose NAM supplementation compared to placebo.

234   Specifically, changes in retinal ganglion cell function as measured by the Vmax ratio,

235   which is a ratio of the saturating amplitude for the PhNR and b-wave (PhNR/b-wave)

236   were analysed. Our lab has worked extensively to improve the repeatability and clinical

237   utility of the PhNR measurement with the specific aim to measure changes to retinal

238   function in a short period.[5,17,18,36] Due to the short-term nature of the study (12 weeks per

239   treatment arm), VF indices were considered as a secondary outcome. Secondary

240   objectives included changes to the PhNR Vmax, VF indices (mean deviation, MD;

241   pattern standard deviation, PSD; mean light sensitivity, MLS), RNFL thickness, IOP and

242   mean arterial pressure (MAP).

243   **Data analysis**

244   ERG waveforms were processed as described previously.[18] In brief, custom-written

245   Matlab® scripts (R2018b, Mathworks, MA, USA) were used to automatically batch

246   process the data. ERG waveforms were processed by an assessor masked to participant

247   characteristics, treatment group and timepoint of measurement. First, a bandpass filter

248   (0.3 – 300 Hz) was applied. The raw data was detrended with a 3rd order polynomial,

249   which was shown to provide the most robust PhNR signal.[18] Amplitudes and implicit

250   times of the a-wave, b-wave (from a-wave trough to b-wave peak) and PhNR (minimum

251   from baseline to trough) were

252   extracted.

253   Amplitudes of the b-wave and PhNR (Figure 3B) across the luminance series were

254   modelled using a saturating hyperbolic function,[37] defined as $V(I) = Vmax*I^n/(I^n + K^n)$,

255   where V (μV) is the amplitude as a function of luminous exposure (I), Vmax the saturating

256   amplitude (μV), K the semi-saturation constant (1/K is the sensitivity) and n the slope

257   (Figure 3C-D). For the PhNR, n was fixed at 1.2 (consistent with our pilot data, and

258   similar to others).[38,39] Further, the PhNR Vmax was analysed as a ratio to the b-wave

259   Vmax (Vmax ratio), which can account for intersession differences to the b-wave.
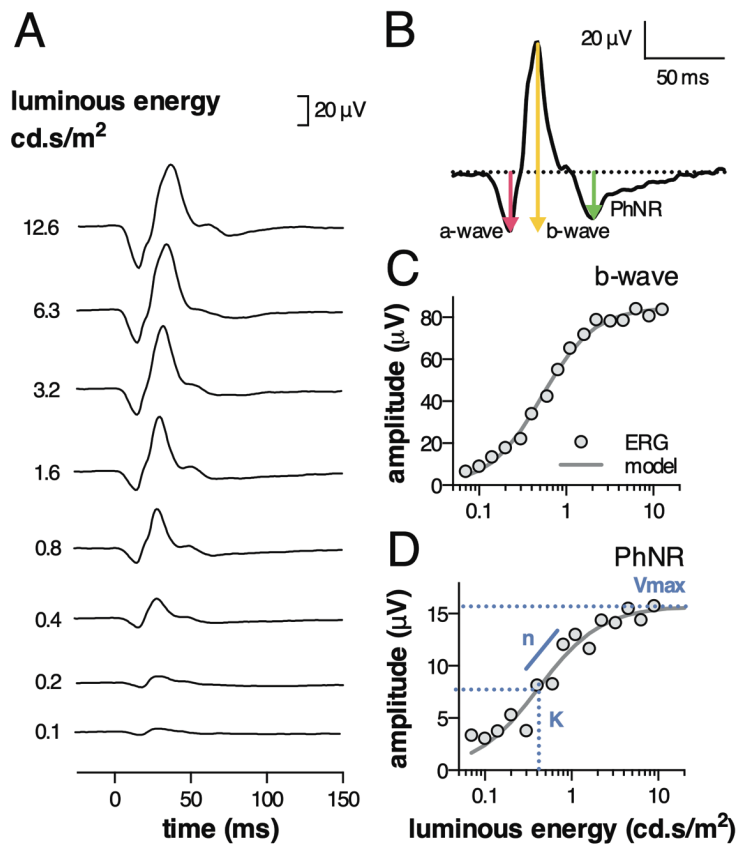
260

Figure 3. Electroretinogram (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).*

In addition to VF MD and PSD, the mean light sensitivity (MLS) was calculated as the average of all perimetric sensitivity values on a 24-2 visual field (excluding one point above and below the blind spot). Visual field parameters are conventionally expressed in logarithmic decibel (dB) scale, however, these were converted to a linear scale (1/lambert) where, $dB = 10 \cdot \log(1/lambert)$ before correlating with other measures.[40,41]

273   The proportion of participants with a ≥1 dB change in MD and PSD in NAM and placebo

274   groups were determined and compared using Fisher's exact test.

275   **Statistical Analysis**

276   Data from one eye per participant were included in the analyses. Placebo and NAM

277   treatments were randomly assigned a label of "Treatment A" or "Treatment B." All

278   analyses were performed in a masked fashion. The biostatistician was given deidentified

279   data with the aforementioned labels and remained masked during the period of data

280   analysis. Prior to study commencement, sample size was calculated based on previous

281   work investigating short-term changes in PhNR Vmax ratio following IOP reduction in

282   glaucoma, where an effect size of 0.56 was found in response to a 30% IOP reduction

283   from baseline (under review). Assuming an attrition rate of 20%, it was estimated that 48

284   eyes were required to achieve 80% power to detect a difference between treatment

285   periods for Vmax ratio and two-sided hypothesis test with an α level of 0.05.

286   The primary analysis set included participants who attended all study visits. Statistical

287   analyses were conducted with the statistician masked to intervention using Stata (SE

288   version 15.1, Texas, US). Treatment adherence was compared between randomisation

289   groups using the Wilcoxon rank-sum test. As a washout period was not utilised, a pre-

290   test for carryover effects was conducted for each parameter.[42] The within-participant sum

291   of values for each parameter was calculated by adding change from baseline values

292   observed under placebo to those under NAM and compared between randomisation

293   groups using two-sample t-test. To test for a period effect, the within participant

294   difference (NAM-PL) was compared between treatment groups using linear regression,

295   adjusting for baseline values of each parameter. The treatment effect for each parameter

296   was assessed following the high-dose period by examining the magnitude and direction

297   of the within-participant difference via linear regression, with adjustment for mean-

298   centred baseline values. Values were analysed in original units of measurement. If a

299   statistically significant treatment effect was found at 12-weeks for any endpoint, then the

300   6-week timepoint was also assessed. Changes in PhNR parameters were also

301   compared to the 95% coefficient of repeatability (COR) established from our previous

302   work where COR was 27% and 28% for PhNR Vmax and Vmax ratio respectively.[17]

303

304   Missing ERG values were associated with fatigue or persistent muscle twitch during

305   recordings that could not be ameliorated with post-hoc signal processing. Values were

306   multiply imputed (25 imputed datasets) using fully conditional specification (chained

307   equations) with a univariate linear regression imputation model to reduce bias. In

308   addition to the imputation variables, imputation models included demographics, clinical

309   data and study group. As a sensitivity analysis, complete-case analyses were performed

310   using the per-protocol set (n = 43) which only included participants who had non-missing

311   data. Results are shown as mean and [95% confidence interval], unless stated otherwise.

312

313   **Results**

314   **Participants**

315   Among 94 participants assessed after medical record screening, 57 were randomised

316   into the study, of which 49 (86%, 65.5±10.0 years, 39% female) completed the study.

317   The participant flow chart is presented in Figure 2 and baseline characteristics of

318   participants in the primary analysis in Table 1. There were no differences in type of

319   glaucoma therapy between treatment groups prior to, or during the study.
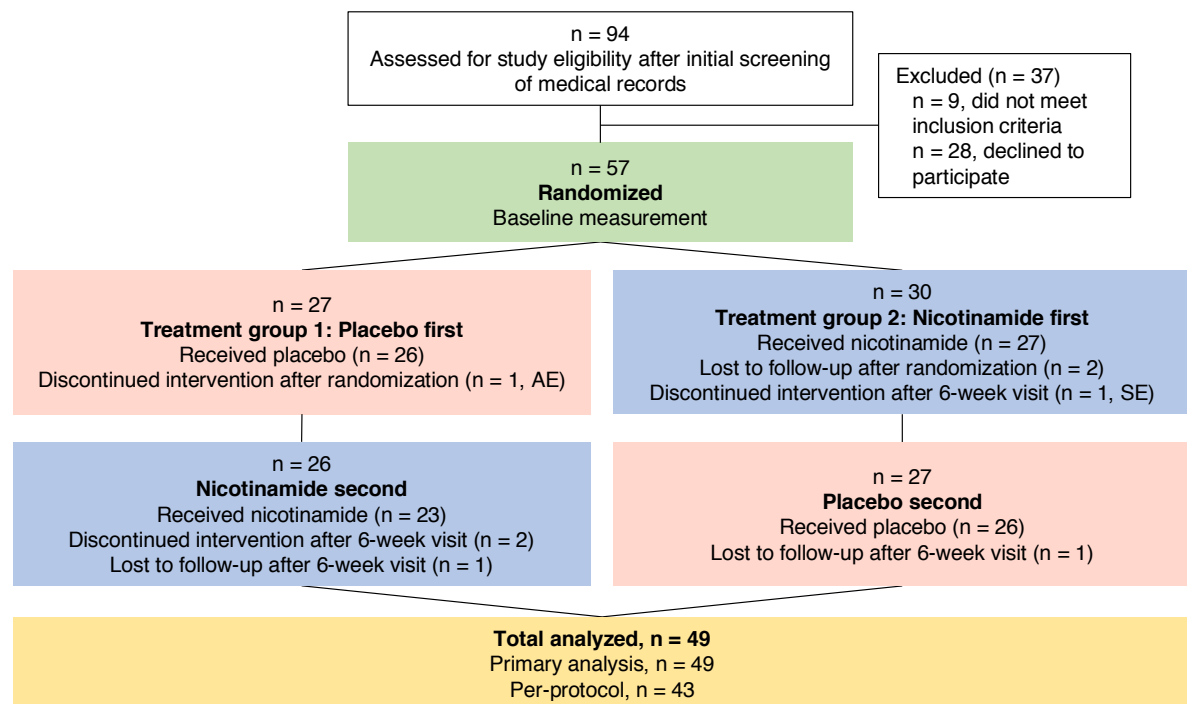
320

321

Figure 4. CONSORT diagram. *Participants were randomised to receive placebo first or nicotinamide first. 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 missing data on outcome variables (n = 6). Participants lost to follow-up (n = 4), participants discontinuing intervention (n = 4). AE – adverse event, SE – side effect.*

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337 ***Table 1. Baseline characteristics for participants in the primary analysis by***

338 ***treatment group (n = 49).***

	Placebo first (n = 23)	Nicotinamide first (n = 26)
	n (%)	n (%)
Sex		
Female	10 (43%)	8 (31%)
Male	13 (57%)	18 (69%)
Eye tested		
Left	11 (48%)	16 (62%)
Right	12 (52%)	10 (38%)
Presence of IOL		
No	12 (52%)	14 (54%)
Yes	11 (48%)	12 (46%)
Glaucoma diagnosis		
POAG/NTG	16 (70%)	21 (70%)
CACG	5 (22%)	3 (12%)
PXF	1 (4%)	2 (8%)
PDG	1 (4%)	0 (0%)
	Mean (SD)	**Mean (SD)**
Age (years)	67.01 (2.05)	65.30 (2.14)
Visual acuity (logMAR)	0.01 (0.14)	-0.01 (0.11)
Intraocular pressure (mmHg)	13.6 (2.7)	14.5 (3.3)
ERG b-wave fitting parameter: n[†]	1.32 (0.16)	1.29 (0.17)
ERG b-wave Vmax (μV) [†]	108.87 (27.48)	99.00 (29.75)
ERG b-wave fitting parameter: 1/K[†]	1.22 (0.30)	1.12 (0.41)
	Median (Q1, Q3)	**Median (Q1, Q3)**
Mean arterial pressure (mmHg) [†]	92.17 (85.00, 96.00)	95.67 (88.17, 98.67)
ERG PhNR fitting parameter: 1/K[†]	2.40 (1.24, 3.64)	1.64 (1.27, 2.68)
ERG PhNR Vmax (μV) [†]	15.30 (11.10, 20.58)	11.39 (9.31, 13.49)
PhNR Vmax/b-wave Vmax ratio[†]	0.14 (0.11, 0.20)	0.12 (0.10, 0.14)
Visual field MD (24-2) dB	-5.46 (-7.20, -1.10)	-4.51 (-8.37, -1.84)
Visual field PSD (24-2) dB	6.77 (2.37, 9.85)	5.89 (3.06, 9.28)

[†]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).
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.

339

340 Majority of participants had primary open angle glaucoma (POAG, 70%), then chronic

341 angle closure glaucoma (16%). Adherence rates were high for both NAM and placebo,

342 with >94% adherence to NAM, demonstrating the high tolerability of both low and high-

343   dose NAM supplementation (Table 2). Only two participants (3%) were non-adherent on

344   NAM and removed from analysis after failing to attend all study visits.

345

346   **Table 2. Distribution of days from baseline to follow-up for participants included**

347   **in the primary analysis and adherence rates (%) at each visit.** *Treatment group 1*

348   *received placebo (PL) first, Treatment group received nicotinamide (NAM) first. P-values*

349   *derived from Wilcoxon rank-sum test to test for differences in adherence between groups,*

350   *n = 49 in total.*

Visit no.	Treatment group	Days from baseline			Adherence (%)		
		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]	0.80
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]	0.70
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]	0.89
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]	0.46

NAM – nicotinamide, PL – placebo, Q1 – lower quartile, Q3 – upper quartile, SD – standard deviation

351

352 **Improvement in Retinal Ganglion Cell Function Following Nicotinamide Treatment**

353 At the 12-week visit, significant improvements in inner retinal function were found in the high-

354 dose NAM arm, with 23% (PhNR Vmax) and 21% (Vmax ratio) of the group improving beyond

355 the respective 95% COR for each measure. Fewer participants on placebo demonstrated

356 improvement (PhNR Vmax: 9%; Vmax ratio: 14%). Some participants deteriorated beyond the

357 COR (9% on placebo, 7% on NAM had a reduced Vmax; 5% on placebo, 2% on NAM had a

358 reduced Vmax ratio). A significant difference in overall treatment effect was found for both

359 Vmax ratio (NAM-PL: 0.01 [0.002, 0.025], $p = 0.02$) and PhNR Vmax (NAM-PL: 1.35 μV [0.159,

360 2.551], $p = 0.03$, Figure 5A-B, Supplementary Table 1). PhNR sensitivity (1/K) did not change

361 with treatment ($p = 0.41$). Overall there was 12.6% [5.0, 20.2] ($p = 0.002$, Figure 5C)

362 improvement in Vmax ratio between baseline and 12-weeks following NAM compared to 3.6%

363 [-3.4, 10.5] ($p = 0.30$) with placebo (between group difference 9.0%, $p = 0.03$). PhNR Vmax

364 improved by 14.8% [2.8, 26.9] ($p = 0.02$, Figure 5D) on NAM, compared to 5.2% [-4.2, 14.6]

365 ($p = 0.27$) on placebo (between group difference 9.6%, $p = 0.04$). There was no evidence of

366 carryover or period effects (Supplementary Tables 1 & 2) and inference was unchanged in the

367 sensitivity analyses of the complete-case set after omitting participants with missing ERG data

368 (Supplementary Table 2). In addition, no significant changes were noted at 6-weeks with low-

369 dose NAM or placebo. There was no evidence of a correlation between the PhNR treatment

370 effect and age, sex, or BMI (Supplementary Figure 1).

371

372 In addition, there was no evidence of a treatment effect on ERG measures of outer retinal

373 function including the a-wave (photoreceptoral function, Figure 5E-F) and b-wave (bipolar cell

374 function, Figure 5G-H) amplitudes or implicit times between NAM and placebo. No differences

375 were found for any b-wave fitting parameters between NAM and placebo groups following the

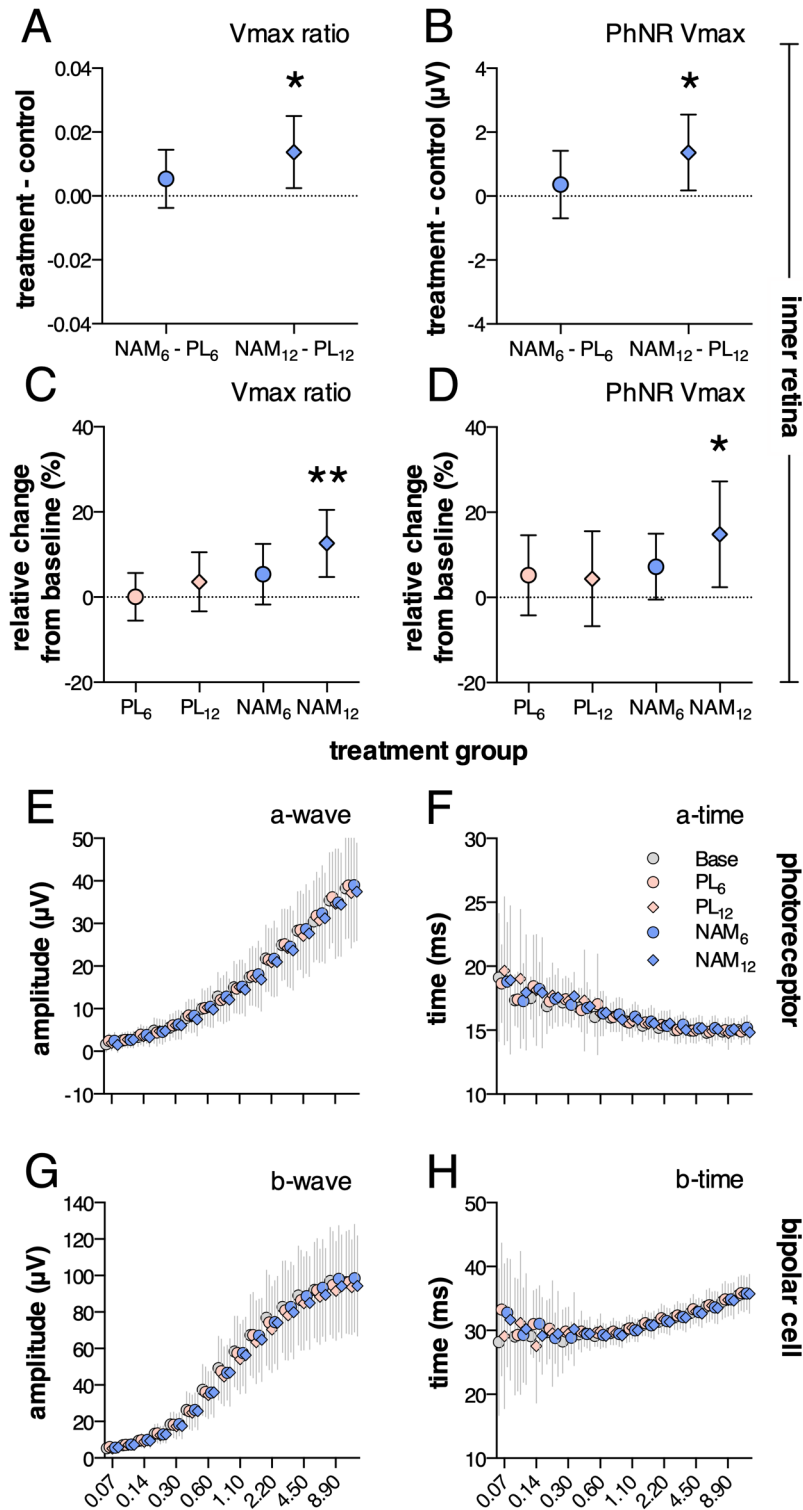376 low or high-dose periods (Supplementary Table 1).

377

Figure 5. Significant improvement in the photopic negative response (inner retinal

function) at 12-weeks post nicotinamide intervention. *A. Difference in Vmax ratio between*

nicotinamide (NAM) and placebo (PL) at 6-weeks (NAM$_6$ – PL$_6$, circles) and 12-weeks (NAM$_{12}$

382 *– PL$_{12}$, diamonds) showing significant increase at NAM$_{12}$ (p = 0.02), B. PhNR Vmax (μV)*

383 *showing a significant increase at NAM$_{12}$ (p = 0.03), C. Vmax ratio expressed as relative*

384 *change from baseline (%) for nicotinamide (blue) and placebo (pink) at 6 and 12-weeks, with*

385 *a significant change at NAM$_{12}$ (p = 0.002, one sample t-test), D. PhNR Vmax (NAM$_{12}$, p =*

386 *0.02), E-F. Luminance-response series for the a-wave and b-wave. No significant difference*

387 *in a-wave amplitude and implicit time (photoreceptoral) following placebo and nicotinamide*

388 *treatment after low dose (6-weeks, circles) or high dose periods (12-weeks, diamonds). G-H.*

389 *No significant change in b-wave amplitudes and implicit times (bipolar cell) following treatment;*

390 *mean ± 95% CI, n = 43. Base – baseline, NAM$_6$ – nicotinamide at 6-weeks, NAM$_{12}$ –*

391 *nicotinamide at 12-weeks, PL$_6$ – placebo at 6-weeks, PL$_{12}$ – placebo at 12-weeks.*

392

393 **Perimetric Changes with Nicotinamide**

394 There were no significant differences in global VF indices between treatment groups

395 (Supplementary Table 1). After 12 weeks of NAM, average MD was no different to placebo

396 (NAM-PL: 0.10 dB [-0.33, 0.53], p = 0.63, Figure 6A). Similarly, PSD reduced only slightly with

397 NAM (NAM-PL: -0.25 dB [-0.63, 0.14], p = 0.20, Figure 6B). Changes in MD and PSD scores

398 for each individual are shown in Figure 6A-B. A greater proportion of participants had an

399 increase in MD by ≥1 dB from baseline following high-dose NAM compared to placebo (27%

400 vs. 16%) and fewer patients taking high-dose NAM had a decrease by ≥1 dB compared to

401 placebo (4% vs 12%, *p = 0.02,* Figure 6C). This was not evident for PSD, where a similar

402 proportion of participants had an increase or decrease by ≥1 dB following NAM and PL (p =

403 0.61, Figure 6D). A moderate positive correlation was found between changes in PhNR Vmax

404 and VF MD at 12 weeks (Figure 6E-F) for both placebo (*Pearson's r* = 0.34, [0.05, 0.57], p =

405 0.02) and NAM treatment (*Pearson's r* = 0.50, [0.24, 0.70], p = 0.0006). There was also

406 evidence of a moderate positive correlation between PhNR Vmax and VF MLS (Figure 6G-H)

407   with placebo (*Pearson's r* = 0.37, [0.09, 0.60], *p* = 0.01) and NAM (*Pearson's r* = 0.54, [0.28,

408   0.72], *p* = 0.0002).
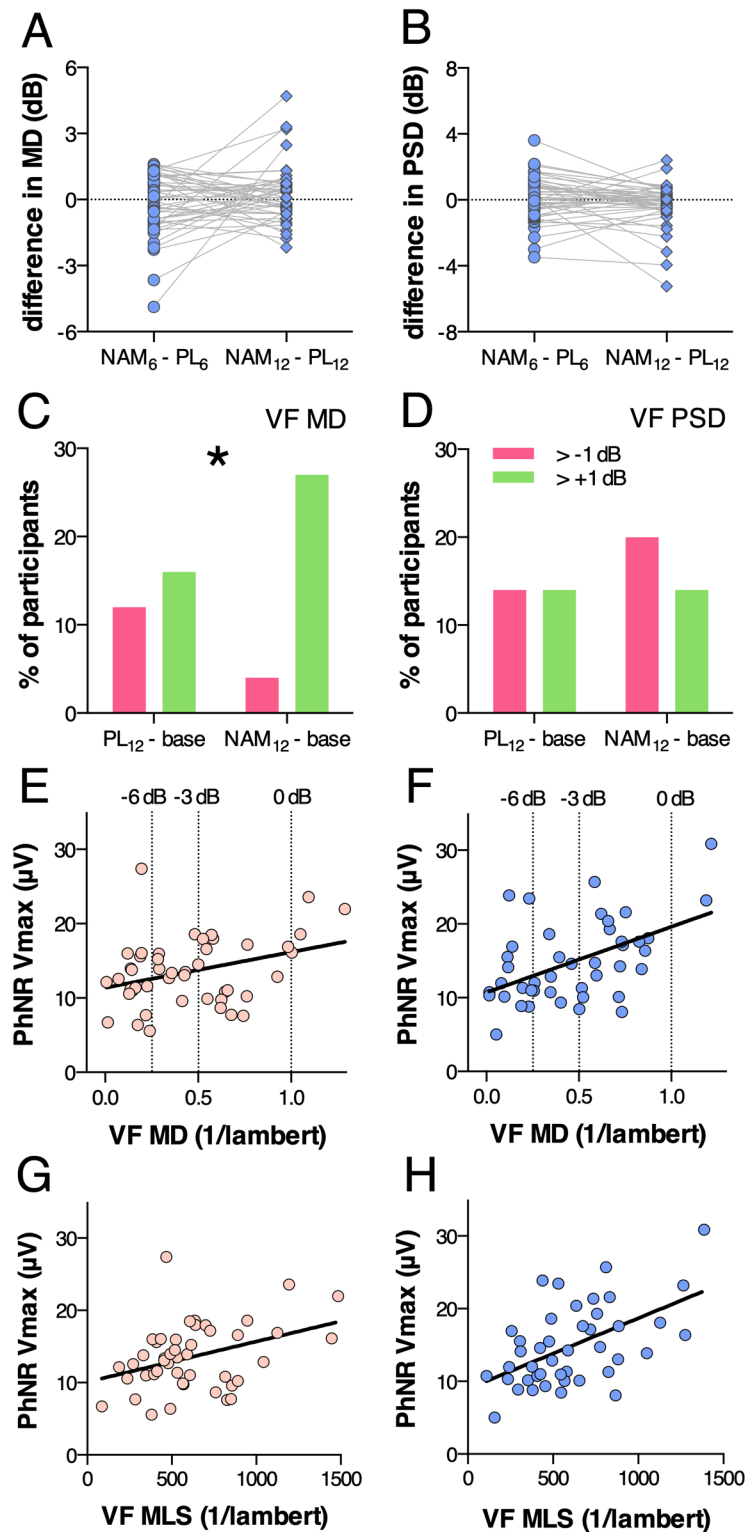
409

410   **Figure 6. Changes in visual field (VF) parameters after treatment.** *A. Time-series plots of*

411   *the difference in VF mean deviation (MD, dB) between nicotinamide (NAM) and placebo (PL)*

412   *groups at 6 and 12- weeks for each individual, B. Difference in VF pattern standard deviation*

413   *(PSD, dB) between NAM and placebo groups at 6 and 12- weeks, C. Proportion of participants*

414   *(%) who demonstrated an improvement (green) or worsening (red) of VF MD by $\geq$ 1 dB*

415   *compared to baseline for NAM and placebo groups (Fisher's exact test, p = 0.02), D.*

416   *Proportion of participants (%) who demonstrated an improvement (green) or worsening (red)*

417   *of VF PSD by $\geq$ 1 dB compared to baseline, E. Linear positive correlation between VF MD*

418   *(1/lambert) and the photopic negative response saturated amplitude (PhNR Vmax, μV) after*

419   *12-weeks of placebo (Pearson's r = 0.34, p = 0.02). Dashed lines show where VF MD in dB*

420   *units (0, -3 and -6 dB) are located, F. Correlation between VF MD (1/lambert) and PhNR Vmax*

421   *after 12-weeks of NAM (Pearson's r = 0.50, p = 0.0006), G. Correlation between VF mean*

422   *light sensitivity (MLS, 1/lambert) and PhNR Vmax after 12-weeks of placebo (Pearson's r =*

423   *0.37, p = 0.01), H. Correlation between VF MLS and PhNR Vmax after 12-weeks of NAM*

424   *(Pearson's r = 0.54, p = 0.0002); n = 49. Base – baseline, NAM_6 – nicotinamide at 6-weeks,*

425   *NAM_{12} – nicotinamide at 12-weeks, PL_6 – placebo at 6-weeks, PL_{12} – placebo at 12-weeks.*

426

427   **IOP and RNFL Thickness Unchanged**

428   There was no evidence of a difference between placebo and NAM in IOP, MAP or VA

429   (Supplementary Table 1). IOP was 13.8 ± 4.1 mmHg (mean ± SD) following NAM compared

430   to 13.4 ± 2.4 mmHg following placebo (mean adjusted NAM-PL: 0.2 mmHg [-0.58, 1.003], *p =*

431   0.59). Overall, there was no change in RNFL thickness. At 12-weeks, the difference in RNFL

432   thickness compared to baseline was, NAM: -0.3 ± 2.9 μm compared to placebo: 0.4 ± 2.4 μm

433   (mean ± SD, *p* = 0.11).

434   **Adverse effects**

435   In this study, high-dose NAM was well tolerated, the most common side effects being mild

436   gastrointestinal discomfort (constipation or soft stools; 10.5%), nausea (5.3%) and headaches

437   (3.5%). In the placebo group, 12.3% of participants reported difficulty in tablet swallowing, and

438   7.0% reported gastrointestinal discomfort. One participant withdrew after experiencing tinnitus

439   whilst on placebo treatment. All symptoms resolved after participants ceased treatment.

440

441

442   **Discussion**

443   We provide evidence that NAM can lead to an early improvement in inner retinal function in a

444   significant proportion of IOP-treated glaucoma patients after 12-weeks of supplementation.

445   The majority of individuals whose PhNR parameters improved beyond 95% COR also

446   improved in VF MD. Overall, NAM was well tolerated with an adherence rate of >94%. As

447   systemic NAM levels have been shown to be reduced in patients with POAG,[28] NAM

448   supplementation may be a convenient, safe, and cost-effective therapy in conjunction with

449   existing IOP-lowering strategies. A longer-term study is being planned to determine whether

450   these functional improvements are sustained and associated with delayed glaucoma

451   progression.

452

453   There is growing evidence that impairments in visual function that occur in experimental and

454   clinical glaucoma may recover following IOP-lowering[5,6,43-45] or in response to the provision of

455   bioenergetic substrates.[7,46] A number of reports have shown short-term improvement in

456   contrast sensitivity and VF parameters in response to IOP-lowering,[6,45,47] and some studies

457   indicate that these improvements in visual function are sustained for extended periods.[48]

458   Gandolfi et al showed significant improvements in contrast sensitivity 3-9 months after

459   trabeculectomy, which persisted for three years.[6] Caprioli and colleagues also demonstrated

460   improved sensitivity on a point-wise VF analysis with long-term improvement in 44% of VF

461   locations, five years after trabeculectomy.[48] Due to the short-term nature of our study, we

462   could perform point-wise analysis. Nevertheless, after 12 weeks of NAM supplementation,

more participants (27%) demonstrated an improvement in VF MD, and fewer (4%)

deteriorated compared to the placebo group. Studies have also provided evidence of ERG

improvement following glaucoma treatment. Improvements in pattern electroretinography

were seen 3-months post-trabeculectomy[43] and following oral acetazolamide.[44] Niyadurupola

et al demonstrated increased PhNR amplitude following a >25% IOP reduction.[5] There is less

evidence supporting visual recovery by means other than IOP-lowering. Casson and

colleagues demonstrated that elevating vitreous glucose levels temporarily improved contrast

sensitivity in pseudophakic individuals with POAG, in response to elevations in vitreous

glucose levels.[7] In the present study, oral supplementation with the metabolic substrate, NAM,

was associated with a 14.8% [2.8%, 26.9%] improvement in PhNR Vmax amplitude and

12.6% [5.0%, 20.2%] improvement in Vmax ratio. Importantly, these effects were independent

of IOP. This raises the prospect that oral NAM supplementation may serve as an adjunct to

IOP-lowering therapies.

NAD^+ depletion is observed in many tissues with advancing age[49] and serum levels of NAM

decline in aged POAG patients.[28] The mechanisms by which NAD^+ may improve RGC function

are not known but work in animal models suggest that maintenance of mitochondrial integrity

and function is a key factor.[27,29,50,51] High-dose NAM supplementation in DBA/2J glaucoma-

model mice prevented RGC soma loss, RNFL thinning, age-related transcriptional and

structural changes in mitochondria and preserved RGC function as measured with PERG.[29,50]

NAM was protective as a prophylactic and therapeutic intervention in a dose-dependent

manner. A similar level of neuroprotection was observed with the overexpression of NAD^+

biosynthetic enzyme, *Nmnat1* in RGCs.[29] A synergistic effect was found with gene therapy

and NAM. Human clinical studies have not previously examined the effect of NAM

supplementation on visual function in glaucoma.

489   NAM is the amide form of vitamin B_3 yet is erroneously referred to as vitamin B_3 in much of

490   the scientific and medical literature. In these contexts, vitamin B_3 encapsulates multiple

491   compounds including niacin/nicotinic acid, which have known ocular and systemic side effects

492   at high doses.[52] However, NAM supplements are widely commercially available and are

493   relatively safe. The most common adverse effects reported in prior clinical studies of high-

494   dose NAM (1.5-6 g/day) were skin flushing and nausea ($\leq$1.5%).[31] Longitudinal studies will

495   reveal the tolerability of long-term high-dose NAM supplementation.

496

497   We enrolled participants already treated for glaucoma with well-controlled IOP. As IOP was

498   unchanged by oral NAM treatment, the observed effect of NAM on inner retinal function is

499   unlikely to be mediated through changes in IOP. Instead, our findings suggest metabolic

500   rescue of RGCs as a putative mechanism of action. We hypothesise that NAM

501   supplementation may benefit eyes with elevated IOP, which is associated with increased RGC

502   metabolic stress and dysfunction.[2,12] The NAM dosage used in our study was derived

503   empirically from preclinical work and clinical trials of the supplement for other indications.

504   Therefore, it is possible that this dose was sub-optimal and/or that a single dose is not ideal

505   for all, as pharmacokinetics and pharmacodynamics will differ between people.[53] However,

506   we did not observe a correlation between treatment effect and baseline demographics.

507   Although a washout period was not utilised, there was no evidence of carryover effects,

508   potentially due to the short plasma half-life of NAM in humans (3.5 hours after a dose of 25

509   mg/kg).[54]

510

511   For the first time, we have provided evidence that oral NAM supplementation leads to an early

512   and measurable improvement in inner retinal function in glaucoma patients already taking

513   IOP-lowering medication. As a proof-of-concept study, we cannot yet say what impact NAM

514   supplementation may have on a patient's quality of life with glaucoma. Thus, a larger clinical

515   trial of long-term NAM treatment is now warranted to explore whether these effects are

516   sustained and can predict a slowing in glaucoma progression.

517

519

References

1. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology* 2014; **121**: 2081-90.

2. Alqawlaq S, Flanagan JG, Sivak JM. All roads lead to glaucoma: Induced retinal injury cascades contribute to a common neurodegenerative outcome. *Exp Eye Res* 2019; **183**: 88-97.

3. Wiggs JL, Pasquale LR. Genetics of glaucoma. *Hum Mol Genet* 2017; **26**: R21-R7.

4. Bengtsson B, Leske MC, Hyman L, Heijl A. Fluctuation of intraocular pressure and glaucoma progression in the early manifest glaucoma trial. *Ophthalmology* 2007; **114**: 205-9.

5. Niyadurupola N, Luu CD, Nguyen DQ, Geddes K, Tan GX, Wong CC, Tran T, Coote MA, Crowston JG. Intraocular pressure lowering is associated with an increase in the photopic negative response (PhNR) amplitude in glaucoma and ocular hypertensive eyes. *Invest Ophthalmol Vis Sci* 2013; **54**: 1913-9.

6. Gandolfi SA, Cimino L, Sangermani C, Ungaro N, Mora P, Tardini MG. Improvement of spatial contrast sensitivity threshold after surgical reduction of intraocular pressure in unilateral high-tension glaucoma. *Invest Ophthalmol Vis Sci* 2005; **46**: 197-201.

7. Casson RJ, Han G, Ebneter A, Chidlow G, Glihotra J, Newland H, Wood JP. Glucose-induced temporary visual recovery in primary open-angle glaucoma: a double-blind, randomized study. *Ophthalmology* 2014; **121**: 1203-11.

8. Viswanathan S, Frishman LJ, Robson JG, Walters JW. The photopic negative response of the flash electroretinogram in primary open angle glaucoma. *Invest Ophthalmol Vis Sci* 2001; **42**: 514-22.

9. Bui BV, Fortune B. Ganglion cell contributions to the rat full-field electroretinogram. *J Physiol* 2004; **555**: 153-73.

10. Colotto A, Falsini B, Salgarello T, Iarossi G, Galan ME, Scullica L. Photopic negative response of the human ERG: losses associated with glaucomatous damage. *Invest Ophthalmol Vis Sci* 2000; **41**: 2205-11.

548   11.   Zhao D, Wong VHY, Nguyen CTO, Jobling AI, Fletcher EL, Vingrys AJ, Bui BV. Reversibility of

549   Retinal Ganglion Cell Dysfunction From Chronic IOP Elevation. *Invest Ophthalmol Vis Sci* 2019;

550   **60**: 3878-86.

551   12.   Crowston JG, Kong YX, Trounce IA, Dang TM, Fahy ET, Bui BV, Morrison JC, Chrysostomou

552   V. An acute intraocular pressure challenge to assess retinal ganglion cell injury and recovery

553   in the mouse. *Exp Eye Res* 2015; **141**: 3-8.

554   13.   Fry LE, Fahy E, Chrysostomou V, Hui F, Tang J, van Wijngaarden P, Petrou S, Crowston JG.

555   The coma in glaucoma: Retinal ganglion cell dysfunction and recovery. *Prog Retin Eye Res*

556   2018; **65**: 77-92.

557   14.   Kong YX, van Bergen N, Bui BV, Chrysostomou V, Vingrys AJ, Trounce IA, Crowston JG.

558   Impact of aging and diet restriction on retinal function during and after acute intraocular

559   pressure injury. *Neurobiol Aging* 2012; **33**: 1126 e15-25.

560   15.   Chrysostomou V, Galic S, van Wijngaarden P, Trounce IA, Steinberg GR, Crowston JG.

561   Exercise reverses age-related vulnerability of the retina to injury by preventing complement-

562   mediated synapse elimination via a BDNF-dependent pathway. *Aging Cell* 2016.

563   16.   Crowston JG, Fahy ET, Fry L, Trounce IA, van Wijngaarden P, Petrou S, Chrysostomou V.

564   Targeting retinal ganglion cell recovery. *Eye (Lond)* 2017; **31**: 196-8.

565   17.   Tang J, Hui F, Hadoux X, Sarossy M, van Wijngaarden P, Coote M, Crowston JG. A

566   Comparison of the RETeval Sensor Strip and DTL Electrode for Recording the Photopic

567   Negative Response. *Transl Vis Sci Technol* 2018; **7**: 27.

568   18.   Tang J, Hui F, Coote M, Crowston JG, Hadoux X. Baseline Detrending for the Photopic

569   Negative Response. *Transl Vis Sci Technol* 2018; **7**: 9.

570   19.   Sieving PA, Nino C. Scotopic threshold response (STR) of the human electroretinogram. *Invest*

571   *Ophthalmol Vis Sci* 1988; **29**: 1608-14.

572   20.   Bach M, Brigell MG, Hawlina M, Holder GE, Johnson MA, McCulloch DL, Meigen T,

573   Viswanathan S. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update.

574   *Doc Ophthalmol* 2013; **126**: 1-7.

575   21.   Fivenson EM, Lautrup S, Sun N, Scheibye-Knudsen M, Stevnsner T, Nilsen H, Bohr VA, Fang

576   EF. Mitophagy in neurodegeneration and aging. *Neurochem Int* 2017.

577   22.   Lee S, Sheck L, Crowston JG, Van Bergen NJ, O'Neill EC, O'Hare F, Kong YX, Chrysostomou
578   V, Vincent AL, Trounce IA. Impaired complex-I-linked respiration and ATP synthesis in primary
579   open-angle glaucoma patient lymphoblasts. *Invest Ophthalmol Vis Sci* 2012; **53**: 2431-7.

580   23.   Osborne NN, del Olmo-Aguado S. Maintenance of retinal ganglion cell mitochondrial functions
581   as a neuroprotective strategy in glaucoma. *Curr Opin Pharmacol* 2013; **13**: 16-22.

582   24.   van de Ven RA, Santos D, Haigis MC. Mitochondrial Sirtuins and Molecular Mechanisms of
583   Aging. *Trends Mol Med* 2017; **23**: 320-31.

584   25.   Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS,
585   Uchida K, Yoshino J, Imai SI. Long-Term Administration of Nicotinamide Mononucleotide
586   Mitigates Age-Associated Physiological Decline in Mice. *Cell Metab* 2016; **24**: 795-806.

587   26.   Srivastava S. Emerging therapeutic roles for NAD(+) metabolism in mitochondrial and age-
588   related disorders. *Clin Transl Med* 2016; **5**: 25.

589   27.   Williams PA, Harder JM, John SWM. Glaucoma as a Metabolic Optic Neuropathy: Making the
590   Case for Nicotinamide Treatment in Glaucoma. *J Glaucoma* 2017; **26**: 1161-8.

591   28.   Kouassi Nzoughet J, Chao de la Barca JM, Guehlouz K, Leruez S, Coulbault L, Allouche S,
592   Bocca C, Muller J, Amati-Bonneau P, Gohier P, Bonneau D, Simard G, Milea D, Lenaers G,
593   Procaccio V, Reynier P. Nicotinamide Deficiency in Primary Open-Angle Glaucoma. *Invest*
594   *Ophthalmol Vis Sci* 2019; **60**: 2509-14.

595   29.   Williams PA, Harder JM, Foxworth NE, Cochran KE, Philip VM, Porciatti V, Smithies O, John
596   SW. Vitamin B3 modulates mitochondrial vulnerability and prevents glaucoma in aged mice.
597   *Science* 2017; **355**: 756-60.

598   30.   Chen AC, Martin AJ, Choy B, Fernandez-Penas P, Dalziell RA, McKenzie CA, Scolyer RA,
599   Dhillon HM, Vardy JL, Kricker A, St George G, Chinniah N, Halliday GM, Damian DL. A Phase
600   3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention. *N Engl J Med* 2015;
601   **373**: 1618-26.

602   31.   Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ, Gale EA, European
603   Nicotinamide Diabetes Intervention Trial G. Safety of high-dose nicotinamide: a review.
604   *Diabetologia* 2000; **43**: 1337-45.

605 32. Johnson CA, Keltner JL, Cello KE, Edwards M, Kass MA, Gordon MO, Budenz DL, Gaasterland
606   DE, Werner E, Ocular Hypertension Study G. Baseline visual field characteristics in the ocular
607   hypertension treatment study. *Ophthalmology* 2002; **109**: 432-7.

608 33. Musch DC, Lichter PR, Guire KE, Standardi CL. The Collaborative Initial Glaucoma Treatment
609   Study: study design, methods, and baseline characteristics of enrolled patients. *Ophthalmology*
610   1999; **106**: 653-62.

611 34. Schweier C, Hanson JV, Funk J, Toteberg-Harms M. Repeatability of intraocular pressure
612   measurements with Icare PRO rebound, Tono-Pen AVIA, and Goldmann tonometers in sitting
613   and reclining positions. *BMC Ophthalmol* 2013; **13**: 44.

614 35. Chen M, Zhang L, Xu J, Chen X, Gu Y, Ren Y, Wang K. Comparability of three intraocular
615   pressure measurement: iCare pro rebound, non-contact and Goldmann applanation tonometry
616   in different IOP group. *BMC Ophthalmol* 2019; **19**: 225.

617 36. Hui F, Tang J, Hadoux X, Coote M, Crowston JG. Optimizing a Portable ERG Device for
618   Glaucoma Clinic: The Effect of Interstimulus Frequency on the Photopic Negative Response.
619   *Transl Vis Sci Technol* 2018; **7**: 26.

620 37. Fulton AB, Hansen RM. Scotopic stimulus/response relations of the B-wave of the
621   electroretinogram. *Doc Ophthalmol* 1988; **68**: 293-304.

622 38. Binns AM, Mortlock KE, North RV. The relationship between stimulus intensity and response
623   amplitude for the photopic negative response of the flash electroretinogram. *Doc Ophthalmol*
624   2011; **122**: 39-52.

625 39. Joshi NR, Ly E, Viswanathan S. Intensity response function of the photopic negative response
626   (PhNR): effect of age and test-retest reliability. *Doc Ophthalmol* 2017; **135**: 1-16.

627 40. Gardiner SK, Demirel S, Johnson CA, Swanson WH. Assessment of linear-scale indices for
628   perimetry in terms of progression in early glaucoma. *Vision Res* 2011; **51**: 1801-10.

629 41. Garway-Heath DF, Holder GE, Fitzke FW, Hitchings RA. Relationship between
630   electrophysiological, psychophysical, and anatomical measurements in glaucoma. *Invest*
631   *Ophthalmol Vis Sci* 2002; **43**: 2213-20.

632 42. Wellek S, Blettner M. On the proper use of the crossover design in clinical trials: part 18 of a
633   series on evaluation of scientific publications. *Dtsch Arztebl Int* 2012; **109**: 276-81.

634   43.   Sehi M, Grewal DS, Goodkin ML, Greenfield DS. Reversal of retinal ganglion cell dysfunction
635   after surgical reduction of intraocular pressure. *Ophthalmology* 2010; **117**: 2329-36.

636   44.   Ventura LM, Porciatti V. Restoration of retinal ganglion cell function in early glaucoma after
637   intraocular pressure reduction: a pilot study. *Ophthalmology* 2005; **112**: 20-7.

638   45.   Evans DW, Hosking SL, Gherghel D, Bartlett JD. Contrast sensitivity improves after brimonidine
639   therapy in primary open angle glaucoma: a case for neuroprotection. *Br J Ophthalmol* 2003;
640   **87**: 1463-5.

641   46.   Shibeeb O, Chidlow G, Han G, Wood JP, Casson RJ. Effect of subconjunctival glucose on
642   retinal ganglion cell survival in experimental retinal ischaemia and contrast sensitivity in human
643   glaucoma. *Clin Exp Ophthalmol* 2016; **44**: 24-32.

644   47.   Wright TM, Goharian I, Gardiner SK, Sehi M, Greenfield DS. Short-term enhancement of visual
645   field sensitivity in glaucomatous eyes following surgical intraocular pressure reduction. *Am J*
646   *Ophthalmol* 2015; **159**: 378-85 e1.

647   48.   Caprioli J, de Leon JM, Azarbod P, Chen A, Morales E, Nouri-Mahdavi K, Coleman A, Yu F,
648   Afifi A. Trabeculectomy Can Improve Long-Term Visual Function in Glaucoma. *Ophthalmology*
649   2016; **123**: 117-28.

650   49.   Katsyuba E, Auwerx J. Modulating NAD+ metabolism, from bench to bedside. *EMBO J* 2017.

651   50.   Williams PA, Harder JM, Cardozo BH, Foxworth NE, John SWM. Nicotinamide treatment
652   robustly protects from inherited mouse glaucoma. *Commun Integr Biol* 2018; **11**: e1356956.

653   51.   Kamel K, Farrell M, O'Brien C. Mitochondrial dysfunction in ocular disease: Focus on glaucoma.
654   *Mitochondrion* 2017; **35**: 44-53.

655   52.   Domanico D, Verboschi F, Altimari S, Zompatori L, Vingolo EM. Ocular Effects of Niacin: A
656   Review of the Literature. *Med Hypothesis Discov Innov Ophthalmol* 2015; **4**: 64-71.

657   53.   Schwartz JB. The current state of knowledge on age, sex, and their interactions on clinical
658   pharmacology. *Clinical pharmacology and therapeutics* 2007; **82**: 87-96.

659   54.   Petley A, Macklin B, Renwick AG, Wilkin TJ. The pharmacokinetics of nicotinamide in humans
660   and rodents. *Diabetes* 1995; **44**: 152-5.

661