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Glucose–induced temporary visual recovery in primary open–angle glaucoma: a double–blind, randomized crossover study

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Introduction

Glaucoma is a term describing a group of ocular disorders with multi–factorial aetiology united by a clinically characteristic, intraocular pressure–associated optic neuropathy.[1] Pathologically, there is a loss of retinal ganglion cells (RGCs), including their axons, which comprise the retinal nerve fibre layer (RNFL) and optic nerve. The most common subtype is primary open–angle glaucoma (POAG). Intraocular pressure (IOP) reduction is currently the only treatment strategy for POAG, and although this retards the average rate of neurodegeneration,[2] some maximally–treated patients continue to progress, and it remains the world's leading cause of irreversible blindness.

The pathogenesis of POAG remains unclear, but impaired ocular perfusion pressure is a well–documented risk factor,[3, 4] a fact that is consistent with the "vascular theory of glaucoma" and suggesting that energy insufficiency at the optic nerve head and retina is part of the pathogenesis in at least a proportion of individuals with POAG.

We have previously shown that elevated vitreous glucose levels provide robust neuroprotection against an experimental model of acute retinal ischaemia.[5] This finding was consistent with earlier experiments demonstrating a decline in vitreous glucose concentration during periods of retinal ischaemia in rabbits,[6] suggesting that the energy–deprived retina metabolizes glycolytic substrates in the vitreous reservoir, a phenomenon that could potentially be clinically exploited.

We subsequently showed the neuroprotective effect of glucose against prolonged ischaemic retinal injury,[5, 7] and more recently extended the protective effect to a rodent model of glaucomatous optic nerve neurodegeneration.[8] *In vitro* evidence from our laboratory indicates that the mechanism of the protective effect is due to

glycolytic adenosine triphosphate (ATP) production and anti–oxidant generation via glucose entry into the pentose phosphate pathway (unpublished data).

In the experimental models and clinical POAG, we conceptualize RGCs as existing in a tripartite population, comprising healthy, "sick" and dead cells.[9] Conceivably, delivery of an energy substrate combined with cellular reducing power to "sick" RGCs may serve to recover function, providing a form of temporary neurorecovery.[9] In the current study, we aimed to demonstrate recovery of a relevant visual psychophysical parameter, contrast sensitivity, as a clinical substrate of neurorecovery in patients with POAG. This study represents a "first to man" attempt to translate our retinal bioenergetics research from the laboratory to the clinic.

Methods

Phase 1 glucose delivery study

Preliminary testing on health volunteers (RJC and AE) indicated that topical 50% glucose was well tolerated and had no discernible effect on the cornea or IOP. The aim of the Phase I study was to determine whether 50% topical glucose delivery could reach the vitreous chamber without adverse effects in either phakic or pseudophakic patients. The study was registered online with the Australian and New Zealand Clinical Trial Registry (ANZCTR; ACTRN12611001225909) The study was designed as a dose escalation trial on non–diabetic patients scheduled for epiretinal membrane peel or macular hole repair by experienced vitreoretinal surgeons (JG and HSN). In the immediate pre–operative period, subjects received 5–minutely drops for 60 minutes. A vitreous sample was taken at the start of surgery (15–30 minutes after the last drop) and immediately sent for analysis of glucose concentration. Initial measurements on 4 control subjects (no topical glucose) found a mean vitreous

glucose concentration of 3·0 mmol (SD 0·28). Allowing for greater variance in a treated group, we estimated that a sample size of 4 treated subjects would provide over 90% power to detect a 25% increase in glucose concentration with an alpha value of 0·05. In addition to this sample, the dose escalation protocol required that the glucose concentration was increased from 10% to 25% to 50%, with two patients receiving a given dose and neither permitted to have any adverse effects before proceeding to the next concentration. Having noted no adverse effects at any concentration, we treated 4 phakic patients and 4 pseudophakic patients with 50% topical glucose. The data were analysed using a regression analysis with glucose concentration as the response variable and group as the predictor. Compared to the control subjects, the vitreous glucose concentration was significantly elevated in pseudophakic ($p = 0·02$) but not phakic patients (Fig. 1).

The effect of topical glucose on visual function (Study 1)

This study was a double–blind, randomized crossover, first–in–man, trial to determine the effect of topical glucose on psychophysical biomarkers in patients with POAG.

Inclusion/exclusion criteria

Non–diabetic pseudophakic patients with definite POAG were recruited from RJC's clinics. Other ocular pathology was an exclusion criterion, but previous glaucoma filtering surgery (at least 12 months prior) or glaucoma medications were permitted. Eyes were required to have at least six 24–2 Humphrey Field Analyzer (Humphrey Instruments, Dublin, CA, USA) field tests with consistent or progressing glaucomatous field defects and a cup to disc ratio of at least 0·8. All eyes had undergone phacoemulsification with "in the bag" intraocular lens (IOL) placement at least 12 months prior to commencement of the study. If both eyes in one individual were eligible then both eyes were included.

Randomization and masking

The study was registered online with ANZCTR (ACTRN12612001134819) and was conducted in the Ophthalmology Department of the Royal Adelaide Hospital. The trial profile is shown in Figure 2. Using a computer–generated random sequence contained in sealed envelopes, eyes were allocated to receive either 50% glucose then 0'9% saline or *vice versa* with a "washout period" of 2–3 weeks. Sterile drops were formulated and dispensed by the hospital pharmacy in coded but identical bottles. Neither the researchers collecting the data nor the patients knew the contents of each bottle. The drops were administered 5 minutely for 1 hour, as per the protocol in the Phase I study. We elected to use 0·9% saline because we felt that highly concentrated saline would not be well tolerated by patients; however, we were aware that the osmolarity was not matched to the glucose (see Study 2 below).

Data collection

Prior to instillation of the drops we recorded the following baseline measurements: the best–corrected logarithm of the minimum angle of resolution (logMAR) acuity of each eye using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart; contrast sensitivity using the CSV–1000 (VectorVision, Ohio,U.S.A.); refraction; IOP; central corneal thickness (CCT); blood glucose; and blood pressure. Age, gender and the average of the most recent 3 mean deviations (MD) from the field test data were also recorded.

The CSV–1000 measures contrast sensitivity at 4 spatial frequencies: 3, 6 12, and 18 cylcles per degree. If the logMAR is > 0·6 (approximately 6/24 Snellen acuity), then contrast sensitivity testing with this method is considered inaccurate and was therefore not performed, as per the manufacturers recommendations (VectorVision, Ohio, U.S.A.). The change in the contrast sensitivity compared to baseline was

recorded in log units as per the manufacturer's recommendations (VectorVision, Ohio, U.S.A.). For eyes with an unrecordable acuity on the ETDRS chart, we assigned a logMAR value 0f 2·00 for "count fingers" and 2·30 for "hand movements". [10]

All measurements were recorded again 15–30 minutes after the instillation of the last drop.

Outcomes

The primary outcome was the change in contrast sensitivity at 12 cycles/degree. Secondary outcomes were change in contrast sensitivity at 3, 6, and 18 cycles/ degree and change in the logMAR acuity.

Ethics

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Human Research Ethics committee at the Royal Adelaide Hospital. All patients gave written informed consent to participate.

Statistical analysis

Exploratory data analyses were performed and a generalized estimating equation (GEE) approach was used for parameter estimation to account for the correlated nature of the data. To overcome the non–normality of the response variables in a data set with a relatively small cluster size, bootstrapping with 1000 replications was used to estimate the variance of the regression parameters, and a Wald test was used for hypothesis testing. A p value $< 0{\cdot}05$ was considered statistically significant; commercially available statistical software was used for the analyses (*Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP)

Role of the funding source

The Ophthalmic Research Institute of Australia had no role in study design, data

collection, analysis or interpretation. The corresponding author and his coauthors had full access to all the data in the study, made a final data interpretation that was unbiased by the sponsor, and made the final decision to submit for publication.

Results of Study 1

Baseline data

The mean age of the participants was 77·2 years (SD 7·7) Eyes generally had moderate to severe glaucoma with an average MD of –12·1 (SD 9·8). The mean baseline logMAR was .22 (SD ·33), and the mean baseline contrast sensitivity at 12 cycles/degree was 0·91 (SD ·51) log units.

Effect on contrast sensitivity and acuity

The exploratory data analysis indicated that glucose tended to increase both the log contrast sensitivity at 12 cycles/degree and the logMAR acuity (boxplots of the dataset are shown Fig. 3 and Fig. 4). The GEE regression analysis confirmed that saline caused no change (0·003 log units) from baseline in the contrast sensitivity at 12 cycles/degree. However, glucose significantly increased contrast sensitivity at 12 cycles/degree by 0·26 (95% CI: 0·13 – 0·38) log units ($p < 0.001$; Fig. 5). Saline had a similarly negligible effect at the other spatial frequencies; however, glucose improved the mean contrast sensitivity at 3, 6, and 18 cycles/degree by 0·20 (95% CI: 0·14 – 0·25), 0·17 (95% CI: 0·07 – 0.27), 0.14 (95% CI: –0·01 – 0·28), respectively (Fig. 6).

Saline had no effect on acuity; however, in the regression model, glucose increased the mean logMAR acuity by 0·04 (95% CI: –0·07 – .005; $p = 0·025$)

The effect on IOP, CCT and refraction

The mean baseline IOP was 13·0 mmHg (SD 5·1). The mean baseline CCT was

503·1 μm (SD 70·6). The mean baseline spherical equivalent was –0·93 dioptres (SD 1·81). There was no significant change in any of these parameters after saline or glucose.

The effect of topical glucose on visual function (Study 2)

The Study 1 results suggested a positive effect of glucose on psychophysical biomarkers in patients with POAG. However, the fact that the saline osmolarity was not matched to the glucose raised concerns about the possibility of an optical effect on the cornea. Although we were concerned about a hyperosmolar solution having a detrimental effect on the corneal epithelium, conceivably a hyperosmolar agent may cause relative dehydration of the corneal stroma, potentially leading to improved visual function. To test the hypothesis that the result from study 1 was optically–induced, we invited back participants from study 1 who had responded positively. Based on the treatment effect and variance observed in Study 1, we estimated that a sample size of 9 eyes would provide an 80% power at an alpha value of 0.05 to detect a treatment effect on contrast sensitivity at 12 cycles/degree of the same magnitude as Study 1. We recruited 4 patients from the original study and a further 3 suitable patients (a total of 14 eligible eyes).

The protocol for this repeat study was identical to Study 1 except that patients received 8% saline (osmolarity matched to 50% glucose).

Results of Study 2

Baseline data

The study flow chart is shown in Figure 6. The mean age of the participants was 74·5 years (SD 6·3). The mean baseline logMAR was 0·57 (SD ·92), and the mean baseline contrast sensitivity at 12 cycles/degree was 0·33 (SD 1·2).

Effect on contrast sensitivity and acuity

Exploratory data analyses again indicated that glucose tended to improve both the contrast sensitivity at 12 cycles/degree and the visual acuity (boxplots of the dataset are shown in Figs. 7 and 8). In the GEE analysis, saline caused a small but non–significant mean reduction in contrast sensitivity at 12 cycles/degree (0·10 log units, 95% CI: –0·26 – 0·06). Glucose significantly improved the contrast sensitivity at 12 cycles/degree by 0.86 log units (95% CI: 0·17 – 1.5; p = 0·014). Saline had a small non–significant negative effect at the other spatial frequencies. Glucose significantly improved the mean contrast sensitivity a 6 cycles/degree by 0·28 (95% CI: 0·12 – 0·44) log units, but had no significant effect at 3 or 18 cycles/degree, 0.07 (95% CI:– 0·09 – 0·23) log units and 0·08 (95% CI: –0·09 – 0·26) log units, respectively (Fig. 9).

The effect on IOP, CCT and refraction

The mean baseline IOP was 10·9 mmHg (SD 3·5). The mean baseline CCT was 505·3 μm (SD 36·1). The mean baseline spherical equivalent was –0·93 dioptres (SD 1·49). There was no significant change in any of these parameters after saline or glucose.

Discussion

This study provides one of the first attempts to translate a novel treatment strategy to glaucoma patients using an efficient methodological paradigm, albeit a "lower hanging fruit" than demonstrative neuroprotection.

Neuroprotection is the relative preservation of neurons.[9] It indicates a reduction in the rate of loss of neurons, and by definition is a mathematical function of time. For chronic neurodegenerative diseases like POAG, neuroprotection takes months to years to convincingly demonstrate.[9] This fact has contributed to the poor clinical

translation of neuroprotective research. In contrast, neurorecovery is the complete or partial restoration of a "sick" neuron to structural and/or functional health.[9] It is distinguished by the fact that it can be demonstrated over a short time period.[9] For example, we conceptualize the recovery of vision due to restoration of the retinal circulation following an episode of "grey–out" experienced by pilots during acceleration stress as an example of neurorecovery. Similarly, in 1941, McFarland and Forbes demonstrated glucose–induced recovery of dark adaptation in hypoxic human subjects, which occurred over short time intervals.[11]

Rather than aiming to demonstrate a neuroprotective effect, we elected to demonstrate temporary glucose–induced recovery of visual psycophysical parameters in patients with POAG. Although the notion of sick RGCs in glaucoma is widely discussed, direct evidence for their existence largely comes from experimental models.[12] However, human studies have shown that IOP reduction can recover RGC function, as demonstrated electrophysiologically,[13] and by recovery of contrast sensitivity in glaucoma patients.[14, 15]

Contrast sensitivity refers to the ability to discern between different light (luminance) levels. It is an important aspect of human vision, and is impaired[16, 17] in POAG. Reduction in contrast sensitivity is well correlated with vision–related quality of life in POAG.[18] Furthermore, contrast sensitivity was shown to rapidly but temporarily recover after calcium channel blockade in patients with normal tension glaucoma.[19] The convergence of evidence indicates that contrast sensitivity in glaucoma is most affected at a spatial frequency of 12 cycles/degree Hence, we chose this as our study primary outcome.{Sample, 1991 #370;Gandolfi, 2005 #207}

In experimental animals, the steady–state vitreous glucose concentration is approximately two–thirds of the blood glucose concentration.[20] Hyperglycaemia

causes a corresponding increase in vitreous glucose, which shows a slower rate of decline than blood as normoglycaemia is restored.[20] Although human data describing the relationship between plasma glucose and vitreous glucose concentration is scarce, vitreous glucose is routinely used in forensic medicine as a marker of blood glucose at the time of death.[21] To our knowledge, there is only one previous report on the blood and glucose concentrations in living human subjects.[22] Our own findings from the non–diabetic patients in the initial phase of the study were consistent with this report.

An initial consideration in this study was the delivery method of glucose to the eye. We elected to use topical glucose delivery. Arguably, this is not an efficient method of drug delivery to the vitreous chamber; however, our rationale was that glucose is a small molecule and it was biologically plausible that it would penetrate the ocular coats. Furthermore, we felt the risk/benefit ratio of alternative delivery methods such as intravitreal injection were not acceptable. Theoretically, the vitreous glucose concentration could have been elevated by rendering the subjects hyperglycaemic; however, the physiological effects of hyperglycaemia include IOP reduction; hence, this would have been a problematic confounder. Given that the permeability of hydrophilic glucose was likely to be poor, we aimed to deliver a high concentration and volume to the eye to optimize the chance of it reaching the vitreous.

We initially demonstrated that topical glucose reached the vitreaous in pseudophakic patients. In phakic patients, the crystalline lens may be acting as a physical or metabolic barrier. We then showed that topical glucose temporarily improved the average contrast sensitivity at 12 cycles/degree. This effect was repeated in a follow–up study which matched the osmolarity of the saline control to the glucose.

Although these data indicated that glucose was acting as a “neurorecoverant” at the level of the retina, this study was not strictly designed to test that hypothesis.

However, we plan to test this hypothesis with a similar study but with retinal electrophysiology as the primary endpoint. The study motivates further retinal bioenergetics research which could conceivably trial related energy substrates in a similar methodological paradigm and more efficient methods of drug delivery in a variety of ophthalmic diseases where energy insufficiency may be part of the pathogenesis.

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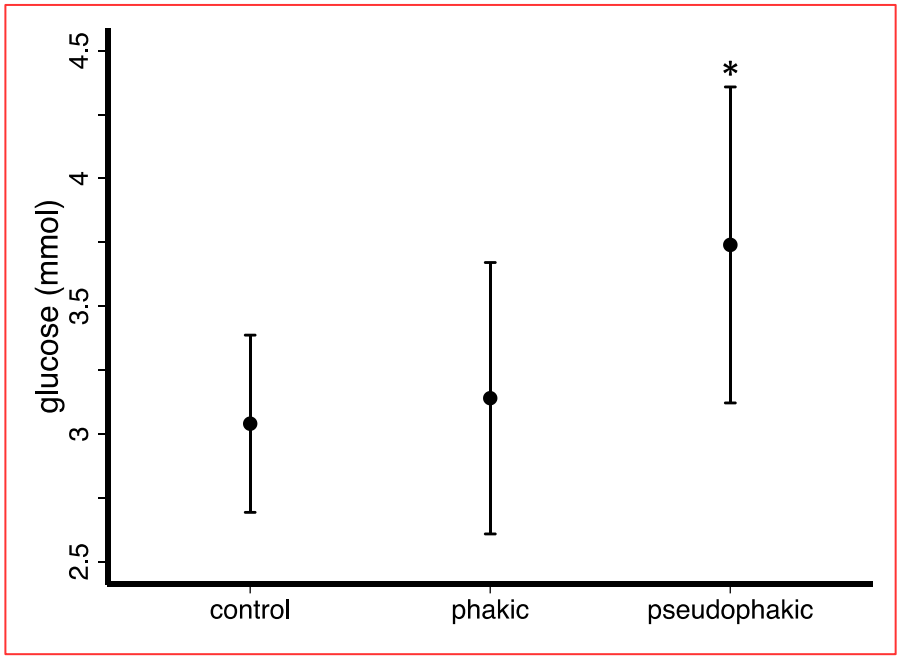
Figure 1

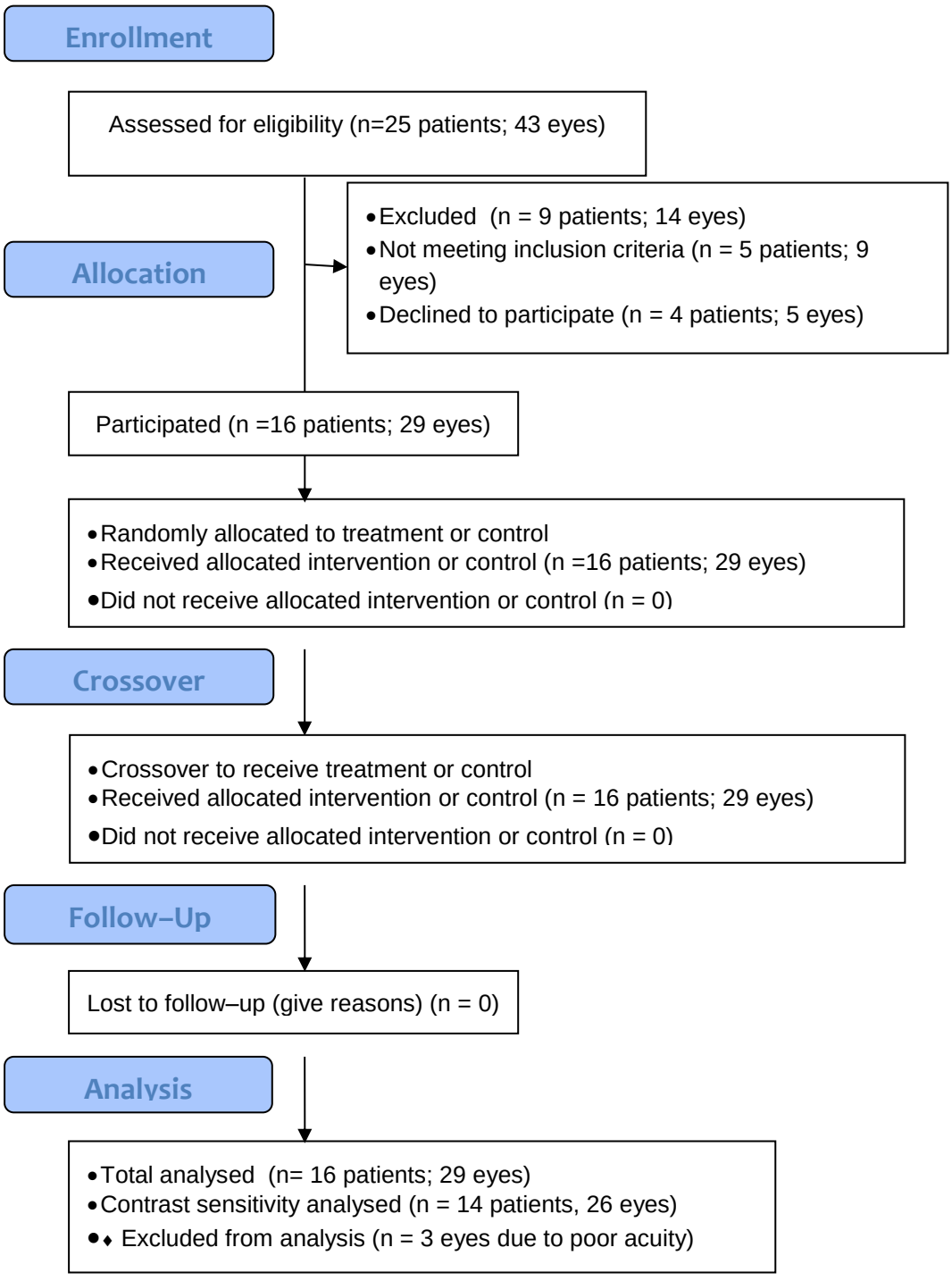


Legend for Figure 1

Mean glucose concentration in the vitreous after 50% topical glucose administration 5–minutely for 60 minutes. *indicates a significant difference compared to the control group (P = 0.02 by t–test); N = 4 for each group; error bars show 95% confidence intervals.)

Figure 2

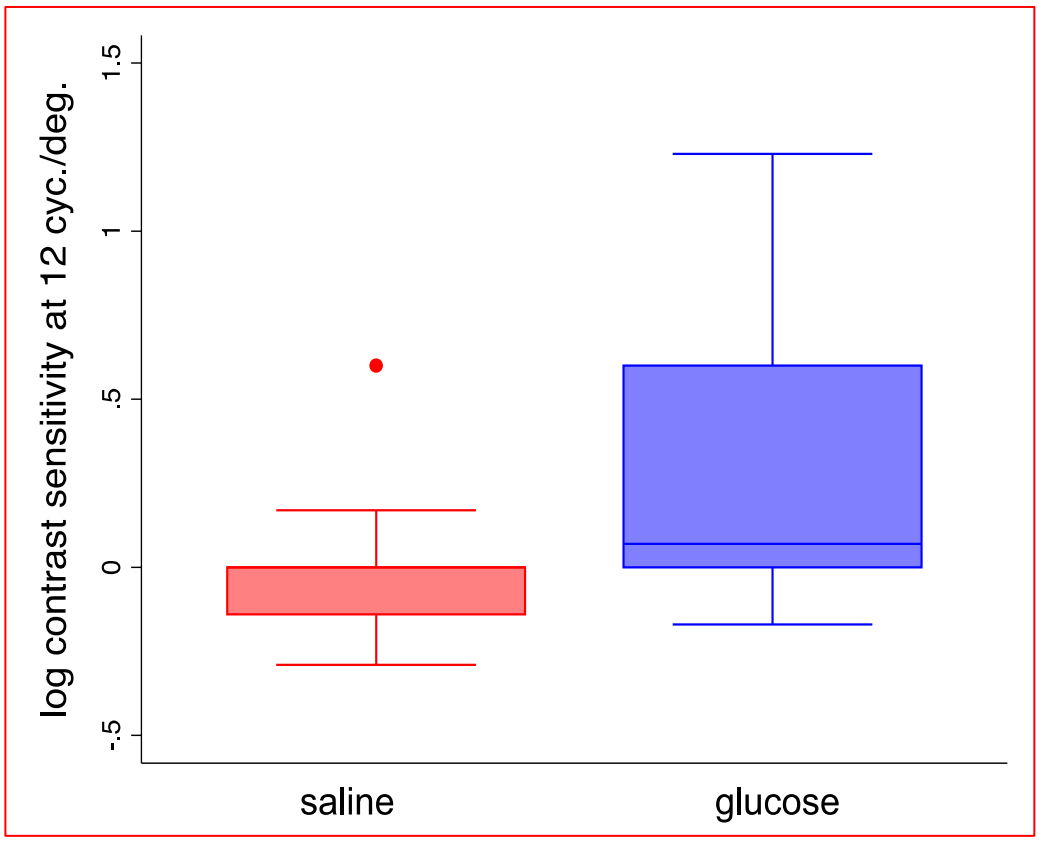
Crossover Design for Study 1



Legend for Figure 2

Flow diagram of Study 1

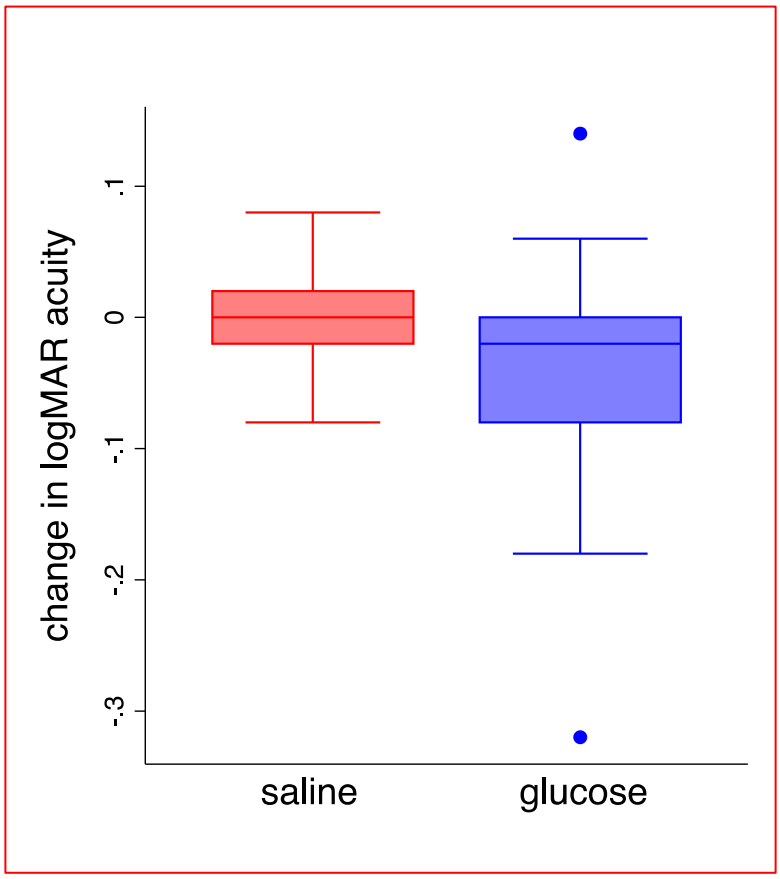
Figure 3



Legend for Figure 3

Box plot showing the change in contrast sensitivity at 12 cycles/degree in the glucose–treated and control groups in Study 1.

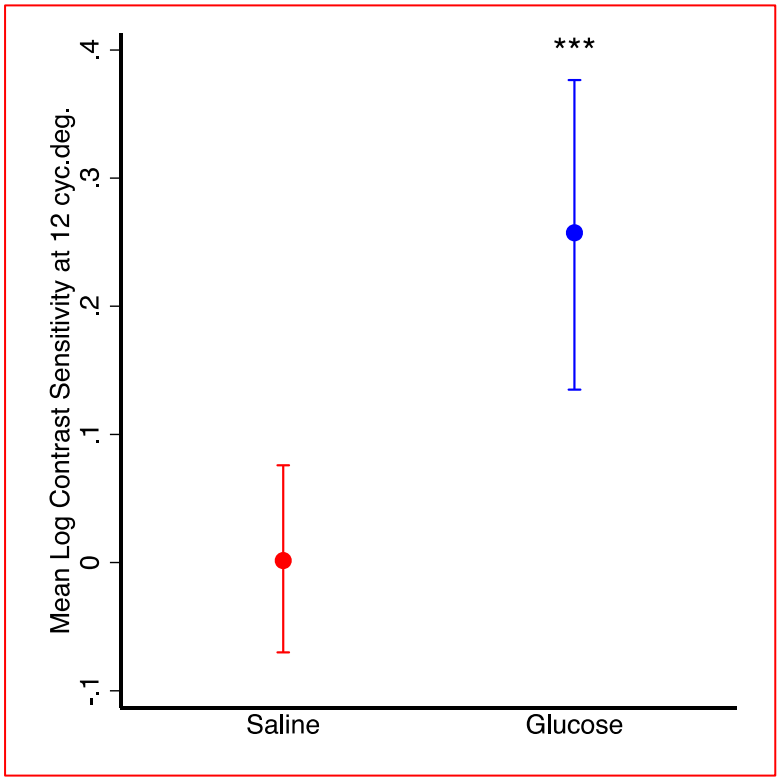
Figure 4



Legend for Figure 4

Box plot showing the change in logMAR acuity in the glucose–treated and control groups in Study 1.

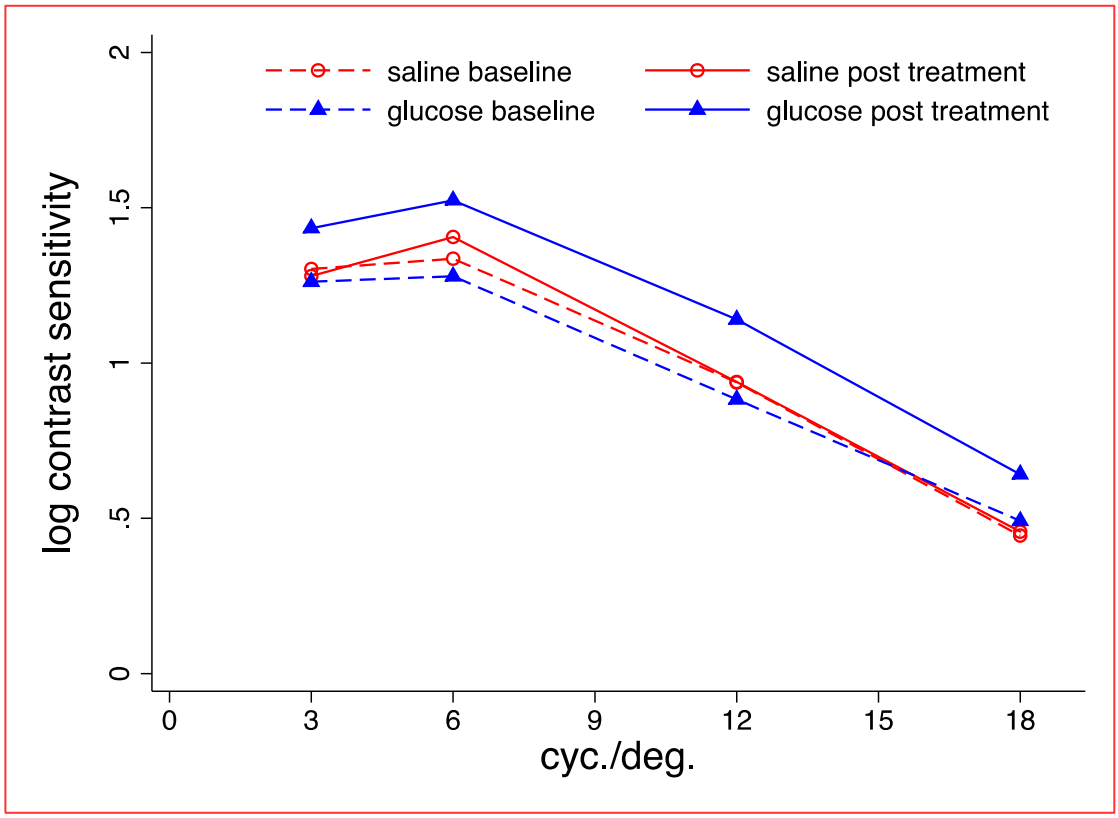
Figure 5



Legend for Figure 5

Glucose significantly increased contrast sensitivity at 12 cycles/degree by 0·26 log units (*** $p < 0.001$; error bars show 95% CIs from GEE regression analysis).

Figure 6

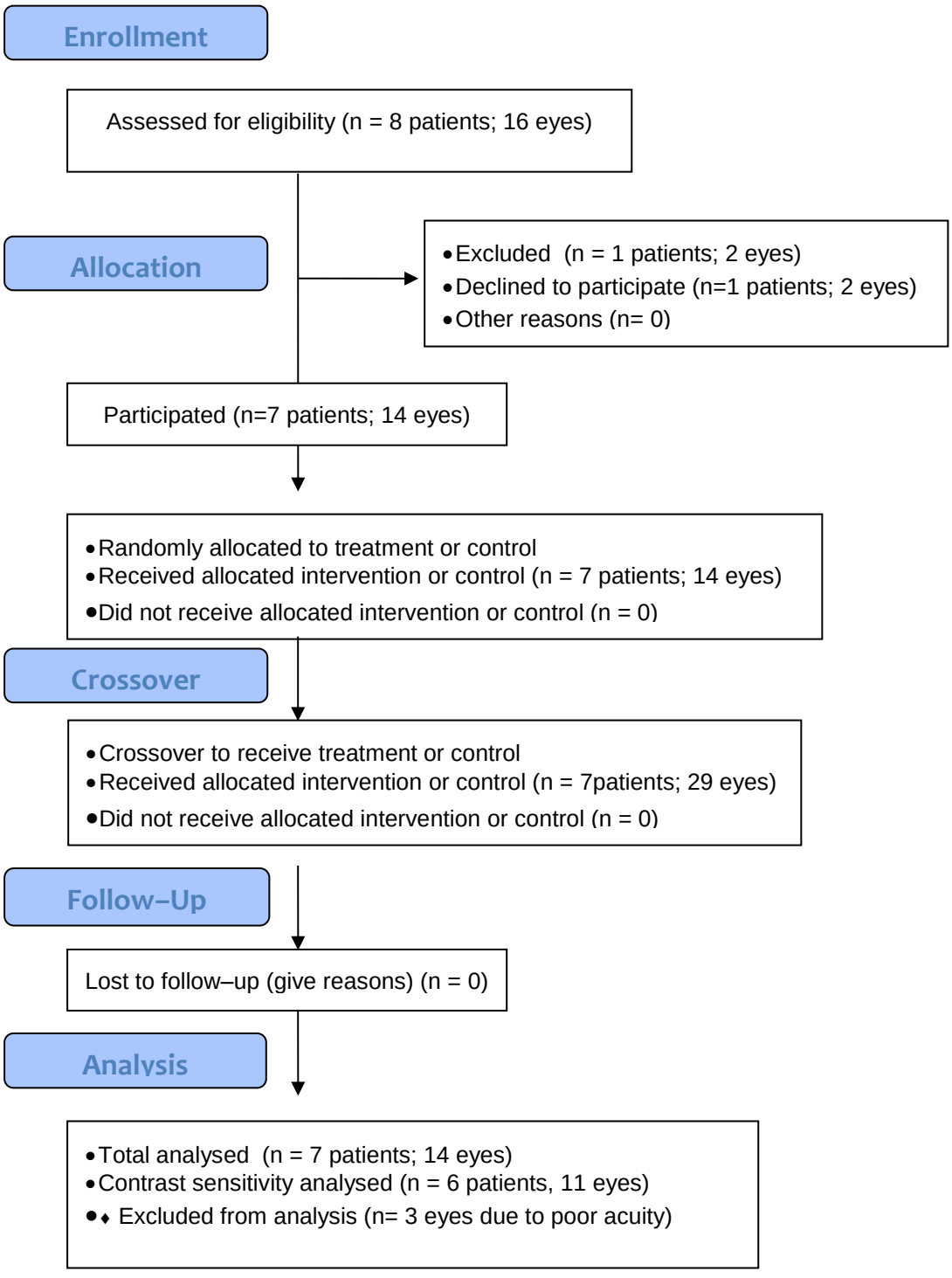


Legend for Figure 6

Log contrast sensitivity at baseline and after glucose or saline control drops in Study 1.

Figure 7

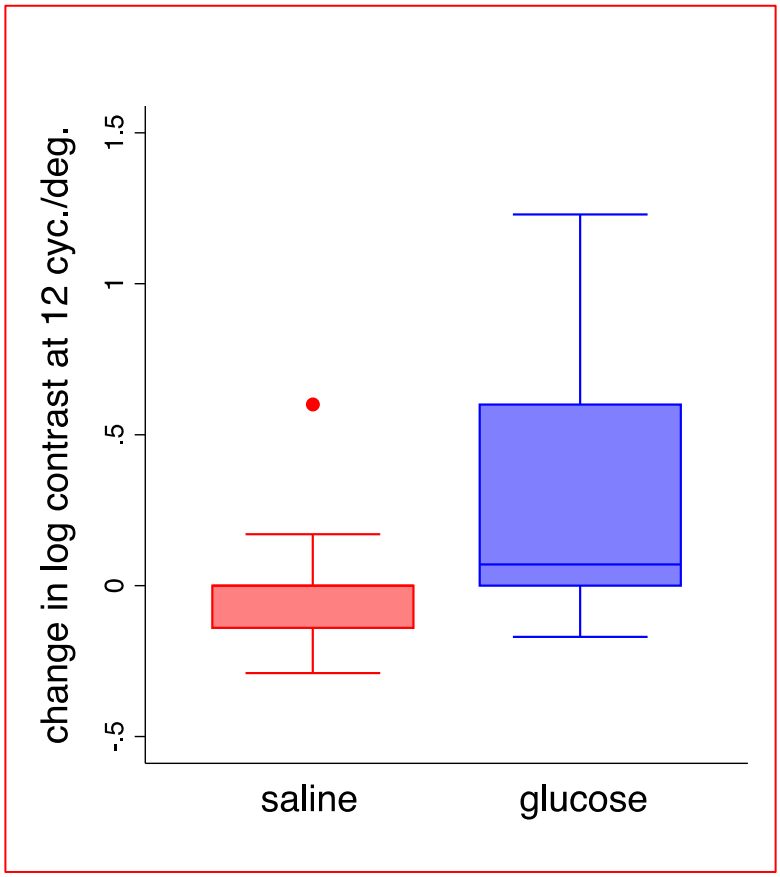
Crossover Design for Study 2



Legend for Figure 7

Flow diagram for Study 2

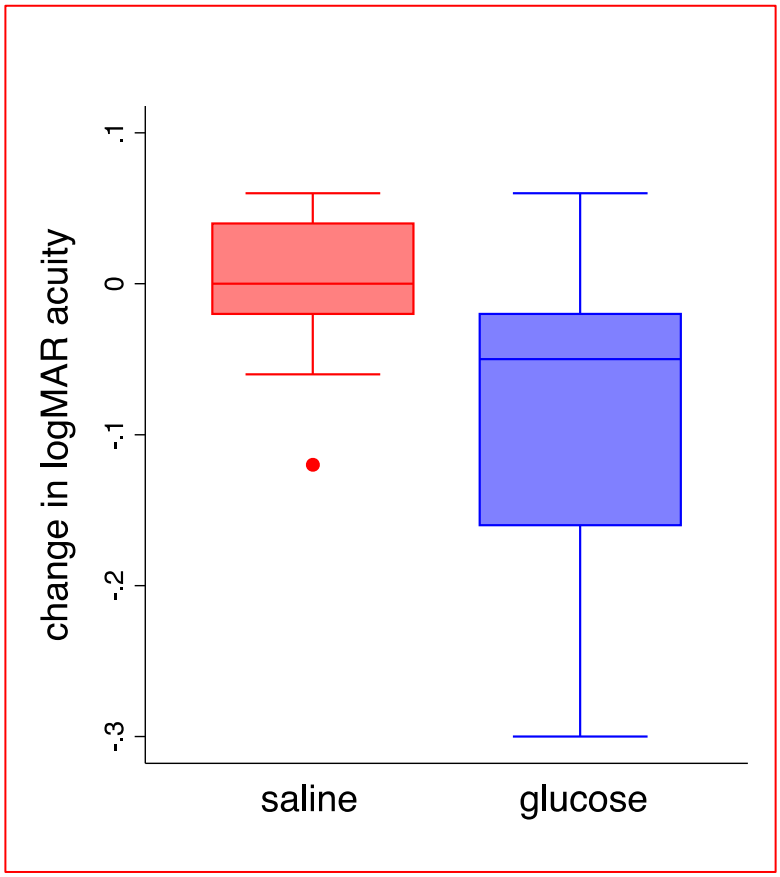
Figure 8



Legend for Figure 8

Box plot showing the change in contrast sensitivity at 12 cycles/degree in the glucose–treated and control groups in Study 2.

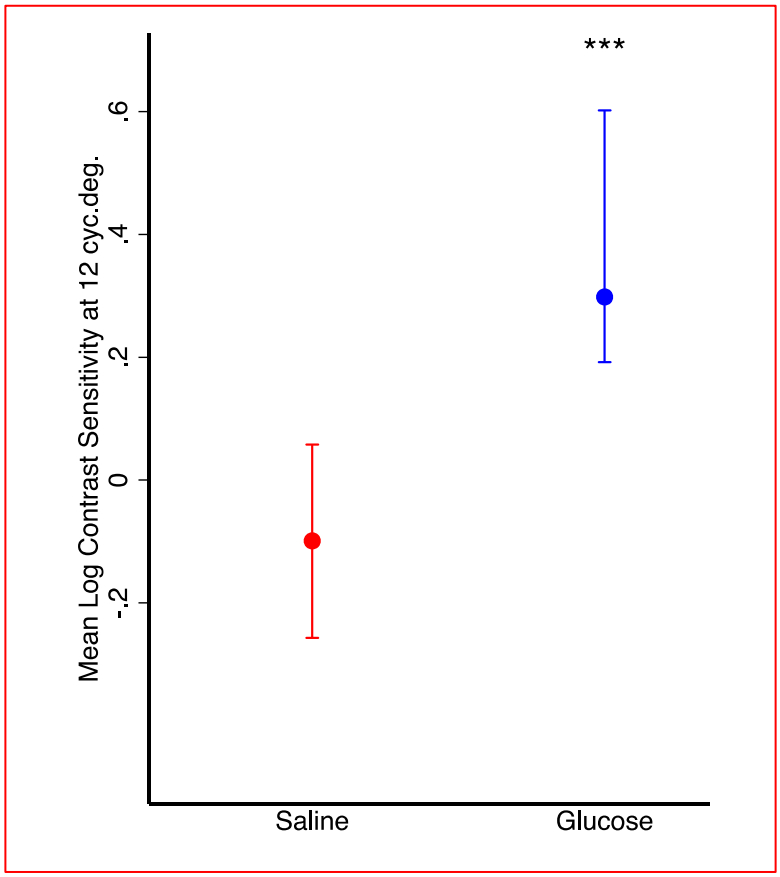
Figure 9



Legend for Figure 9

Box plot showing the change in logMAR acuity in the glucose–treated and control groups in Study 1.

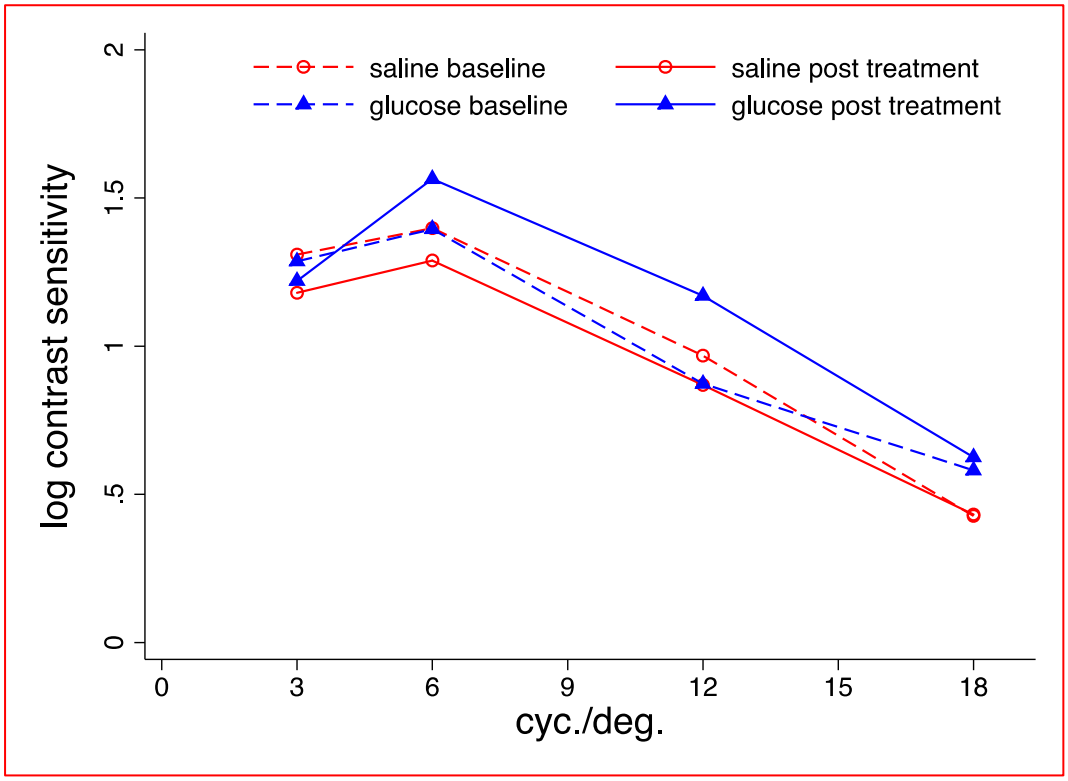
Figure 10



Legend for Figure 10

Glucose significantly improved the mean contrast sensitivity at 12 cycles/degree by 0.30 log units from baseline. ***p = 0.014; error bars show 95% CIs from GEE regression analysis).

Figure 11



Legend for Figure 11

Log contrast sensitivity at baseline and after glucose or saline control drops in Study 2.