SUNY COLLEGE OF OPTOMETRY
GRADUATE CENTER FOR VISION RESEARCH

Effects of *Gingko biloba* on Systemic and Retinal Blood Circulation

By

Durpri Lin, BS

Thesis

In partial satisfaction of the requirements for the degree of

Master of Science in Vision Science

State University of New York

College of Optometry

APRIL, 2023

Approved by Dissertation Committee:

Alexandra Benavente-Perez

Date: 02May2023

Suresh Viswanathan

Date: 05/1/2023

Xiaoying Zhu

Date: 01May2023

Stewart Bloomfield, PhD
Associate Dean, Graduate Studies & Research

Table of Contents

Abstract

Introduction: The use of alternative medicine has increased in recent years due to its minimal side effects and holistic approach to healthcare. Ginkgo biloba extract (GBE) is a natural antioxidant derived from leaves of the Maidenhair tree and is known to improve blood vessel health. However, its effect on the retinal circulation is not fully understood. The purpose of this study is to examine the effect of GBE oral supplements on the retinal circulation.

Methods: Blood pressure (Omron HEM-705CP), intraocular pressure (Canon T2 non-contact Tonometer), and blood flow velocities in the ophthalmic artery, central retinal artery, and short posterior ciliary arteries (Color Doppler imaging, Sequoia) were obtained from participants aged 22 to 36 with good ocular and systemic health. Measurements were performed between 12-5pm to control for circadian rhythm effects at 3 study visits: 1 week before baseline at pre-supplement visit (T_{-1}), at baseline (T_0) and after 4 weeks of 240mg/day GBE supplementation at post-supplement visit (T_4). Ocular perfusion pressure (OPP) was calculated as OPP = 2/3 * (Mean Arterial Pressure – IOP).

Results: Thirteen participants were recruited (5m, 8f; 25.54 ± 3.64 years). No significant changes in systemic blood pressure, OPP or retinal circulation were observed between pre-supplement visit (T_{-1}) and baseline (T_0) prior to GBE supplementation. However, the ophthalmic and short posterior ciliary arteries peak systolic velocities increased from baseline (T_0) to post-treatment (T_4) (ophthalmic artery baseline ave ± SD: 18.97 ± 6.67cm/s; post-treatment:24.33 ± 6.90cm/s; short posterior ciliary artery baseline: 10.56 ± 1.87cm/s; post-treatment: 11.58 ± 1.97cm/s; both $p < 0.05$). The increases in ophthalmic and short posterior ciliary arteries peak systolic velocities did not correlate with changes in systolic BP, diastolic BP, or OPP.

Discussion: Our preliminary data suggests that 240mg/day of Ginkgo biloba extract (GBE) may increase blood flow in two major retinal ocular arteries. Such increase appears independent from changes in systemic blood pressure or OPP.

Introduction

Alternative medicine has become increasingly mainstream in recent years. The main benefits of alternative treatments, such as dietary supplements, are their minimal side effects and holistic approach to healthcare. Herbal products in particular are an important component of the increasing trend toward alternative medicine. One herbal product of current interest is the natural antioxidant Ginkgo biloba.[1]

Ginkgo biloba

Derived from the leaves of the Maidenhair tree, Ginkgo biloba's medicinal properties were first recognized 5000 years ago in ancient China.[2] Recently, the extract of Ginkgo biloba leaves (GBE) has gained popularity as one of the most widely used herbal supplements.[2] Studies on GBE's protective effects have unveiled a variety of molecular mechanisms by which GBE improves blood vessel health. Overall, GBE appears to have major effects on antioxidant capacity, vasomotor function, and platelet activation. Firstly, GBE increases antioxidant activity by scavenging free radicals and increasing the expression of antioxidant genes.[3] Secondly, GBE has vasorelaxant effects by mediating factors released from the endothelium.[4] Thirdly, GBE decreases platelet aggregation by competitively inhibiting platelet activation factor (PAF) from binding its membrane receptor.[5] Additional effects of GBE on signaling pathways, ionic perturbations, cell adhesion, and smooth muscle cell activation have been reported, [1,2] highlighting GBE's potential as an effective broad treatment for many vascular disorders

In vitro studies of Ginkgo biloba

Although Ginkgo biloba is known to improve blood vessel health, its effects on ocular tissue is not fully understood. Evidence in the literature has shown that Gingko biloba has beneficial effects in the eye.[6–8] An in vitro study of light-exposed rats injected with Gingko biloba suggests that Gingko biloba has multiple protective effects in the degenerated retina by decreasing light-induced retinal thinning, scavenging free radicals, reducing the damaged retina's consumption of antioxidative enzymes, and alleviating damage to tissue lipids.[9] Investigation into the retinal function of light-exposed rats also found that Gingko biloba treatment protects against photoreceptor loss and improves retinal function.[10] Another in vitro study proposed that Gingko biloba may exert anti-inflammatory effects in the retina of diabetic rats by reducing levels of the proinflammatory mediators TNF-α and VEGF.[11] Overall, experimental studies of Gingko biloba have shown that this natural antioxidant can reduce free radicals and inflammatory cytokines in the retina, attenuate light-induced retinal thinning, and protect photoreceptor cell function.

Clinical studies of Ginkgo biloba

Clinical studies of GBE in the eye have thus far been mainly focused on its beneficial effects in glaucoma. Three studies have been published on the effects of GBE on blood flow in glaucoma patients. The results of one study found that normal tension glaucoma (NTG) patients who were administered 80mg GBE twice a day for four weeks had significant increases in mean blood flow, volume, and velocity in the peripapillary region.[7] Another study described open angle glaucoma (OAG) patients who were given antioxidant supplements containing 120mg GBE per day for one month to have a

significant increase in blood supply to the orbit, improved retinal capillary perfusion, and decreased vascular resistance.[8] Currently, there are two ongoing clinical trials investigating the effects of GBE supplementation in glaucomatous eyes (*NCT03761992, NCT04334564)* and one ongoing trial exploring the efficacy of GBE supplementation as a treatment for retinitis pigmentosa (*NCT02465749*). Outcome measures in the current studies include retinal blood vessel density, visual field sensitivity, retinal nerve fiber layer thickness, visual acuity, electroretinogram (ERG), and/or retinal vessel oxygen saturation. The diverse array of outcome parameters underscores the scientific community's increasing knowledge of Gingko biloba's potentially widespread protective effects in the eye.

The recent surge in research on Gingko biloba, combined with its reported beneficial effects on blood flow, make GBE a strong candidate as a dietary supplement to maintain the health of ocular tissue by increasing blood circulation. The aim of this Master's research is to examine the effect of GBE oral supplements on the retinal circulation, with the ultimate goal to discover an alternative nutrition-based treatment for promoting ocular health.

Methods

Study Design

The design of this study was a phase 0 prospective, longitudinal, interventional clinical trial. This study was approved by the Institutional Review Board at SUNY College of Optometry. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study (*NCT04448535*).

Human subjects

Inclusion criteria for the study population was healthy adults aged 22 to 36 years, corrected visual acuity of 20/40 or better, and no prior Ginkgo biloba supplementation within the past three months. Exclusion criteria included all significant ocular and any systemic disorders considered likely to affect the ocular or systemic vasculature, history of ocular surgery, any eye or systemic diseases that affected vision, current smokers, and subjects who were pregnant, breastfeeding, or planning conception. Due to GBE's known action of inhibiting platelet activation, subjects currently taking warfarin, aspirin, or any antiplatelet medications were excluded from the study.

Protocol

Subjects participated in 3 study visits (6 hours total) over the span of 5 weeks. During the initial screening at pre-supplement visit (T_{-1}), informed consent and general medical history were obtained from the subject. The researcher confirmed good general health and good ocular health in the subject and performed automated blood pressure (Omron HEM-705CP), intraocular pressure (Canon T2 non-contact Tonometer), ocular

biometry (Lenstar), distance and near visual acuity, and objective refraction (Shin-Nippon NVision-K 5001 autorefractor).

For qualified subjects, measures of ocular vascular hemodynamics were performed using color doppler imaging (CDI) and ocular perfusion pressure (OPP). CDI used ultrasound imaging to assess blood flow velocities such as peak systolic velocity (PSV) and end diastolic velocity (EDV) in the ophthalmic artery (OA), central retinal artery (CRA), and short posterior ciliary arteries (SPCAs). Furthermore, CDI velocities were used to calculate values of resistance index (RI) for each retrobulbar vessel, based on the formula RI = (PSV - EDV) / PSV. OPP represented the difference between arterial blood pressure and intraocular pressure (IOP), and was used to signify the degree of blood perfusion into the eye.

At pre-supplement visit (T_{-1}), initial baseline measurements of blood flow velocities in the ophthalmic artery, central retinal artery, and short posterior ciliary arteries were obtained using CDI. Each measurement of PSV, EDV, and RI was repeated sequentially to assess intra-researcher variability. After one week of no GBE supplementation, the same measurements were repeated at baseline visit (T_0). After one month of 240mg/day GBE supplementation, changes in these same parameters were investigated at post-supplement visit (T_4). A flow diagram of the protocol is shown in Figure 1 and details of the methodology are discussed below.

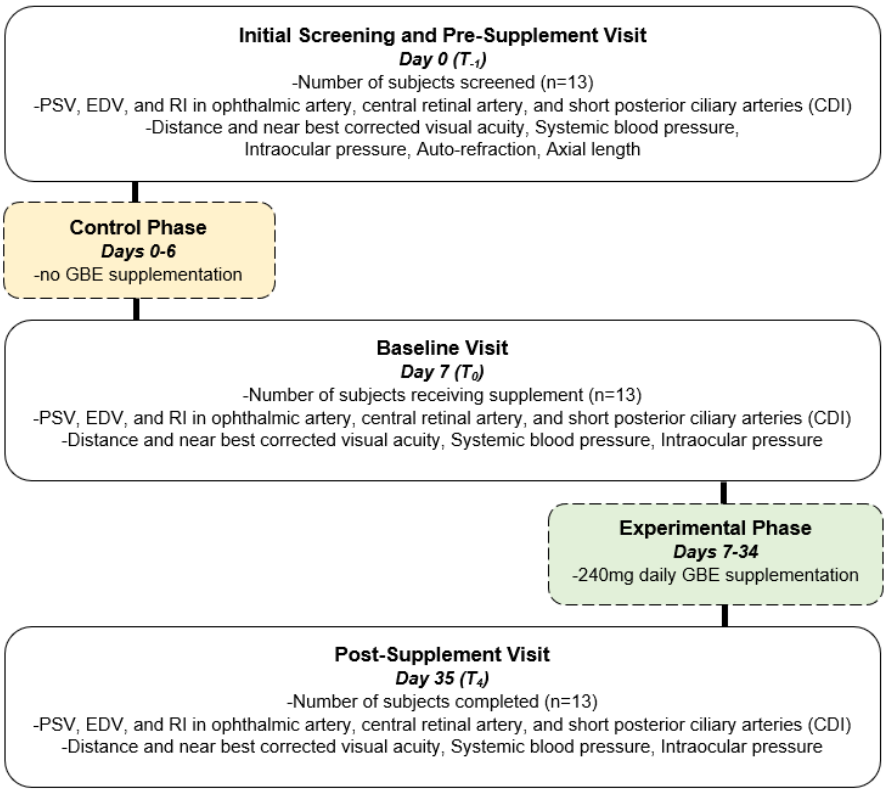

Figure 1. Flow diagram of the protocol for this Phase 0, prospective, longitudinal, interventional clinical trial.

Control phase (days 0-6): At the end of the pre-supplement visit (T_{-1}), subjects were instructed to go home and maintain normal dietary and lifestyle habits for one week without GBE supplementation. The purpose of this first control phase of the study, in which subjects underwent one week without supplementation, was to establish each subject as his/her own control for any observed variations in measurements. One week after the first visit, subjects returned for a baseline visit (T_0).

T_0: Study measurements conducted during the pre-supplement visit (T_{-1}) were repeated at the baseline visit (T_0). Subjects were then assigned one month's supply of GBE supplements. A daily dosage of 240mg GBE was chosen because it is a moderate

dosage lying in between the reported minimum (120mg) and maximum (900mg) daily dosages of Ginkgo biloba in current clinical trials (*NCT02376114, NCT02465749*). Subjects were instructed to consume one tablet of 120mg GBE in the morning and another in the evening, and to continue maintaining normal dietary and lifestyle habits for one month. The purpose of this second phase of the study (experimental phase), in which subjects underwent one month of Ginkgo biloba supplementation, was to determine if GBE consumption would induce any changes in the vascular supply of ocular tissue.

T_4: At one month after the baseline visit (T_0), subjects returned for a post-supplement visit (T_4). Study measurements conducted during the baseline visit (T_0) were repeated at this post-supplement visit.

Color Doppler Imaging

The Siemens ACUSON Sequoia Ultrasound System was used to measure blood flow velocities in the three major retinal ocular arteries. Color Doppler imaging assessed peak systolic velocity (PSV), end diastolic velocity (EDV) and resistance index (RI) in the ophthalmic artery (OA), central retinal artery (CRA), and short posterior ciliary arteries (SPCAs). Two measurements of each parameter were taken sequentially at each visit in order to determine intra-researcher repeatability of CDI.

Ocular perfusion pressure

Ocular perfusion pressure (OPP) was calculated as OPP = 2/3 * (Mean Arterial Pressure – Intraocular pressure). Blood pressure was measured on the same arm at each

visit with the Omron HEM-705CP. Intraocular pressure was measured on the same eye at each visit with the Canon T2 non-contact Tonometer.

Statistical Analysis

A total of three parameters were measured for three arteries. The peak systolic velocity (PSV), end diastolic velocity (EDV), and resistance index (RI) were measured for ophthalmic artery, central retinal artery, and short posterior artery. Each data set was first analyzed for normal distribution using the Kolmogorov-Smirnov Test of Normality ($p<0.05$). Changes in the PSV, EDV, and RI in each of the three major retinal ocular arteries were then analyzed using the t-test for 2 dependent means. Changes from pre-treatment to baseline were analyzed using two-tailed hypothesis ($p<0.05$), and changes from baseline to post-treatment were analyzed using one-tailed hypothesis ($p<0.05$). Correlations between blood flow changes and three factors (axial length, systemic blood pressure, and OPP) were analyzed using the Pearson Correlation Coefficient. Changes in systolic blood pressure, diastolic blood pressure, OPP, and pulse rate were analyzed with the t-test for 2 dependent means, using two-tailed hypothesis ($p<0.05$) for pre-treatment to baseline and one-tailed hypothesis ($p<0.05$) for baseline to post-treatment. Repeatability of CDI blood flow measurements was analyzed on the Bland-Altman Plot.

Results

The results of changes in PSV, EDV, and RI over three study visits for all three retinal arteries are summarized in Table 1. Details of the results are discussed below.

		Pre-supplement visit (T_1)	Baseline visit (T_0)	Post-supplement visit (T_4)
OA	PSV*	20.79 ± 7.71 cm/s	18.97 ± 6.67 cm/s	24.33 ± 6.90 cm/s
	EDV	5.02 ± 2.29 cm/s	5.33 ± 2.41 cm/s	6.42 ± 1.53 cm/s
	RI	0.76 ± 0.06	0.72 ± 0.06	0.73 ± 0.06
CRA	PSV	11.35 ± 3.17 cm/s	11.54 ± 2.40 cm/s	12.32 ± 2.61 cm/s
	EDV	4.05 ± 2.04 cm/s	4.32 ± 1.03 cm/s	4.45 ± 1.11 cm/s
	RI	0.65 ± 0.10	0.62 ± 0.05	0.63 ± 0.07
SPCA	PSV*	9.95 ± 2.14 cm/s	10.56 ± 1.87 cm/s	11.58 ± 1.97 cm/s
	EDV	3.36 ± 1.38 cm/s	4.07 ± 1.34 cm/s	4.25 ± 1.06 cm/s
	RI	0.65 ± 0.10	0.62 ± 0.05	0.63 ± 0.07

Table 1. Summary of PSV, EDV, and RI measurements in three retinal arteries before and after one month of GBE supplementation.

Ophthalmic Artery (OA)

Peak Systolic Velocity

The data for OA PSV was normally distributed with a p-value of 0.32. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 2. The difference between measurements in a single visit were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

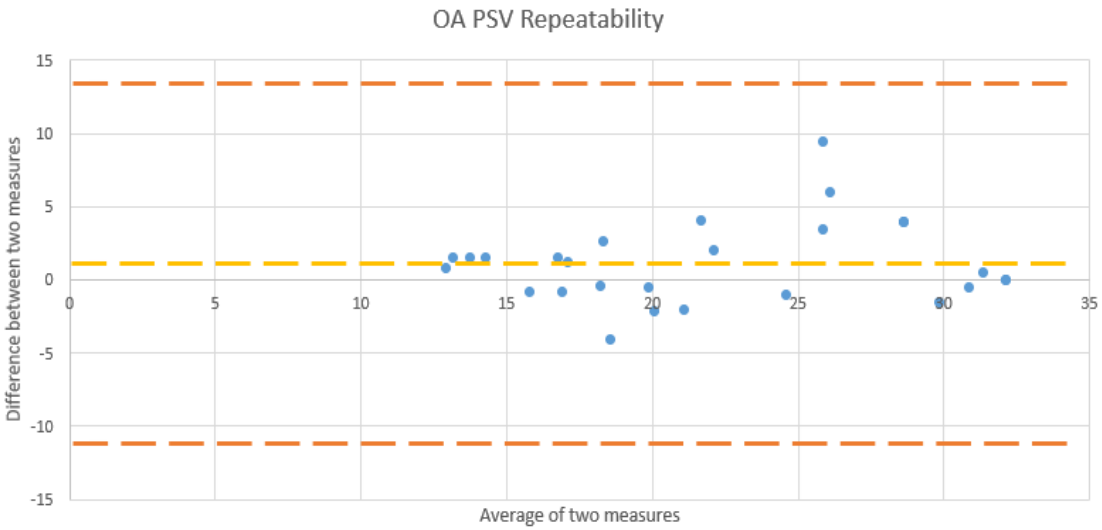

Figure 2. Bland-Altman plot showing good repeatability of OA PSV measurements.

Comparing the pre-treatment control phase OA PSV (T_{-1}) to baseline (T_0), the p-value was 0.33 (pre-treatment ave ± SD: 20.79 ± 7.71cm/s; baseline: 18.97 ± 6.67cm/s). Therefore, the OA PSV remained stable one week apart when participants had no major changes to their diet or physical activity. Comparing the experimental baseline (T_0) to post-treatment (T_4), the p-value was 0.02 (baseline ave ± SD: 18.97 ± 6.67cm/s; post-treatment: 24.33 ± 6.90cm/s). Therefore, the OA PSV significantly increased after one month of 240mg/day ginkgo supplementation, as depicted by the blue line on Figure 3. Individual changes in the OA PSV of all thirteen subjects from pre-treatment (T_{-1}) to baseline (T_0) to post-treatment (T_4), are shown on Figure 4.

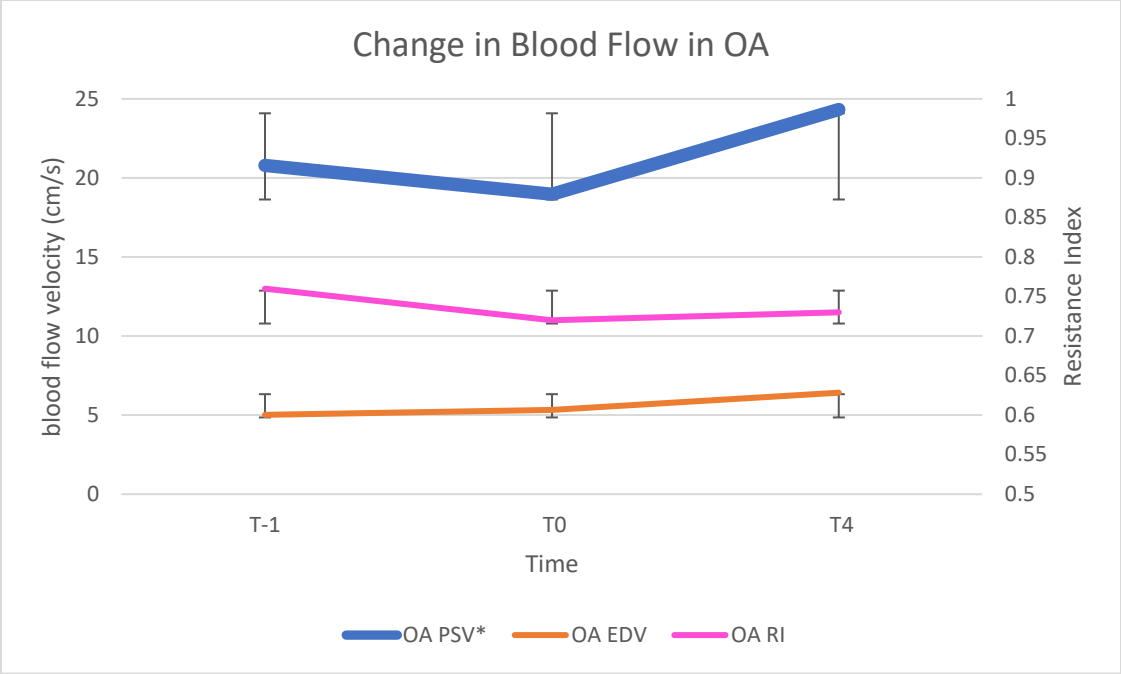

Figure 3. Change in three blood flow parameters in the ophthalmic artery. A significant increase in OA PSV is depicted by the blue line.

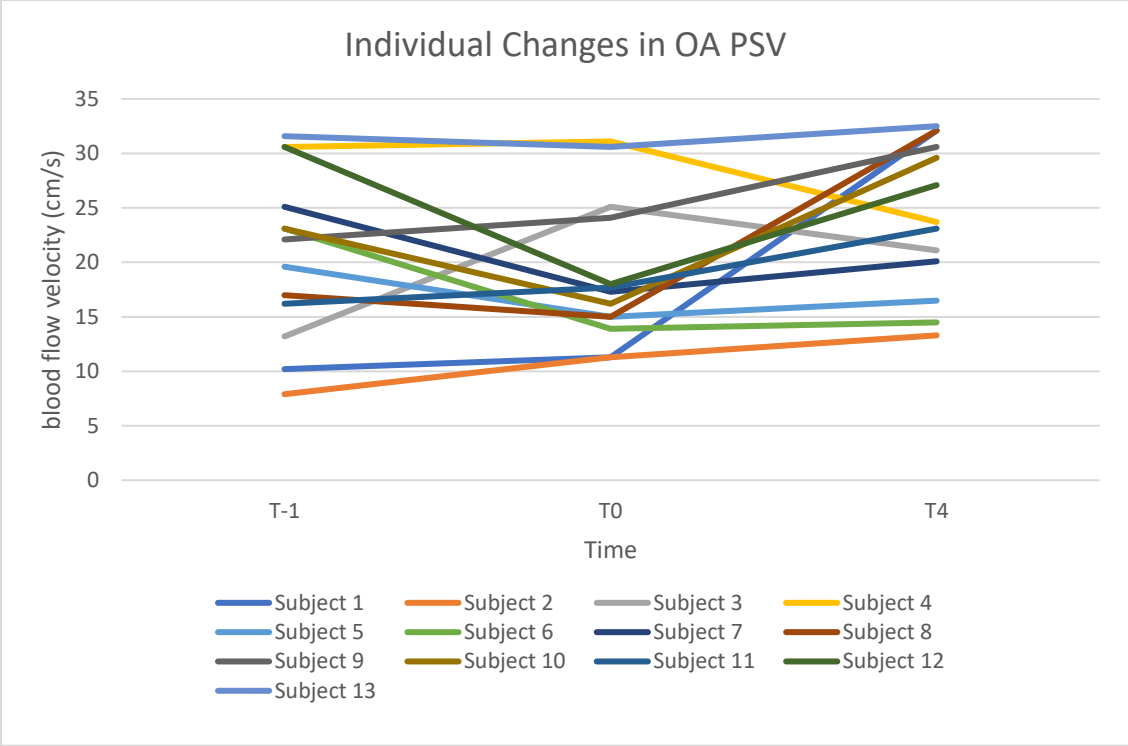

Figure 4. Individual changes in the ophthalmic artery's peak systolic velocity for all thirteen subjects.

There was no correlation between OA PSV and axial length. The Pearson correlation coefficient for axial length and velocity change R-value was 0.40. Therefore only 40% of the PSV increase could be explained by the length of the eyeball. The p-value was 0.17, so this result was not significant.

There was no correlation between OA PSV and systemic blood pressure. The Pearson correlation coefficient for systolic BP and velocity change R-value was 0.03. Therefore only 3% of the PSV increase could be explained by the systolic BP. The p-value was 0.93, so this result was not significant. The Pearson correlation coefficient for diastolic BP and velocity change R-value was 0.17. Therefore only 17% of the PSV increase could be explained by the diastolic BP. The p-value was 0.58, so this result was not significant.

There was no correlation between OA PSV and OPP. The Pearson correlation coefficient for OPP and velocity change R-value was 0.07. Therefore only 7% of the PSV increase could be explained by the OPP. The p-value was 0.85, so this result was not significant.

Ophthalmic Artery

End Diastolic Velocity

The data for OA EDV was normally distributed with a p-value of 0.47. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 5. Most differences between measurements in a single visit were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

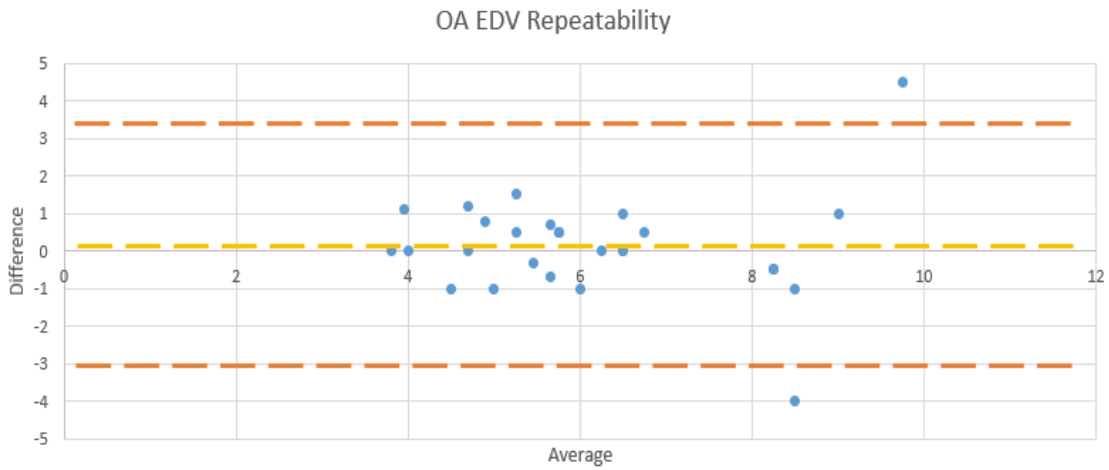

Figure 5. Bland-Altman plot showing good repeatability of OA EDV measurements.

Comparing the pre-treatment control phase (T_{-1}) to baseline (T_0), the p-value was 0.70 (pre-treatment ave ± SD: 5.02 ± 2.29cm/s; baseline: 5.33 ± 2.41cm/s). Therefore, when patients came one week apart without major changes to their diet, the OA EDV remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.10 (baseline ave ± SD: 5.33 ± 2.41cm/s; post-treatment: 6.42 ± 1.53cm/s). Therefore, the OA EDV did not significantly increase after one month of 240mg/day ginkgo supplementation, as depicted by the orange line on Figure 3.

There was no correlation between OA EDV and axial length. The Pearson correlation coefficient for axial length and velocity change R-value was 0.14. Therefore only 14% of the EDV increase could be explained by the length of the eyeball. The p-value was 0.64, so this result was not significant.

There was no correlation between OA EDV and systemic blood pressure. The Pearson correlation coefficient for systolic BP and velocity change R-value was 0.29. Therefore only 29% of the EDV increase could be explained by the systolic BP. The p-

value was 0.34, so this result was not significant. The Pearson correlation coefficient for diastolic BP and velocity change R-value was 0.40. Therefore only 40% of the EDV increase could be explained by the diastolic BP. The p-value was 0.18, so this result was not significant.

There was no correlation between OA EDV and OPP. The Pearson correlation coefficient for OPP and velocity change R-value was 0.28. Therefore only 28% of the EDV increase could be explained by the OPP. The p-value was 0.36, so this result was not significant.

Ophthalmic Artery

Resistance Index

The data for OA RI was normally distributed with a p-value of 0.75. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 6. Most differences between measurements in a single visit were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

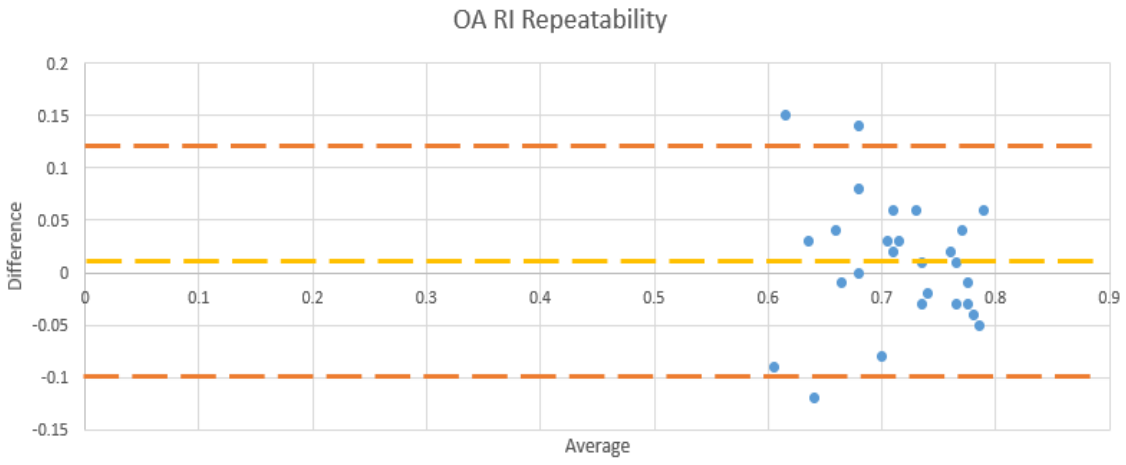

Figure 6. Bland-Altman plot showing good repeatability of OA RI measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.07 (pre-treatment ave ± SD: 0.76 ± 0.06cm/s; baseline: 0.72 ± 0.06cm/s). Therefore, when patients came one week apart without major changes to their diet, the OA RI remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.33 (baseline ave ± SD: 0.72 ± 0.06cm/s; post-treatment: 0.73 ± 0.06cm/s). Therefore, the OA RI did not significantly decrease after one month of 240mg/day ginkgo supplementation.

There was no correlation between OA RI and axial length. The Pearson correlation coefficient for axial length and RI change R-value was 0.29. Therefore only 29% of the RI change could be explained by the length of the eyeball. The p-value was 0.33, so this result was not significant, as depicted by the pink line on Figure 3.

There was no correlation between OA RI and systemic blood pressure. The Pearson correlation coefficient for systolic BP and RI change R-value was 0.27. Therefore only 27% of the RI change could be explained by the systolic BP. The p-value was 0.31, so this result was not significant. The Pearson correlation coefficient for diastolic BP and RI change R-value was 0.31. Therefore only 31% of the RI change could be explained by the diastolic BP. The p-value was 0.30, so this result was not significant.

There was no correlation between OA RI and OPP. The Pearson correlation coefficient for OPP and RI change R-value was 0.14. Therefore only 14% of the RI change could be explained by the OPP. The p-value was 0.64, so this result was not significant.

Central Retinal Artery

Peak Systolic Velocity

The data for CRA PSV was normally distributed with a p-value of 0.95. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 7. The difference between measurements in a single visit were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

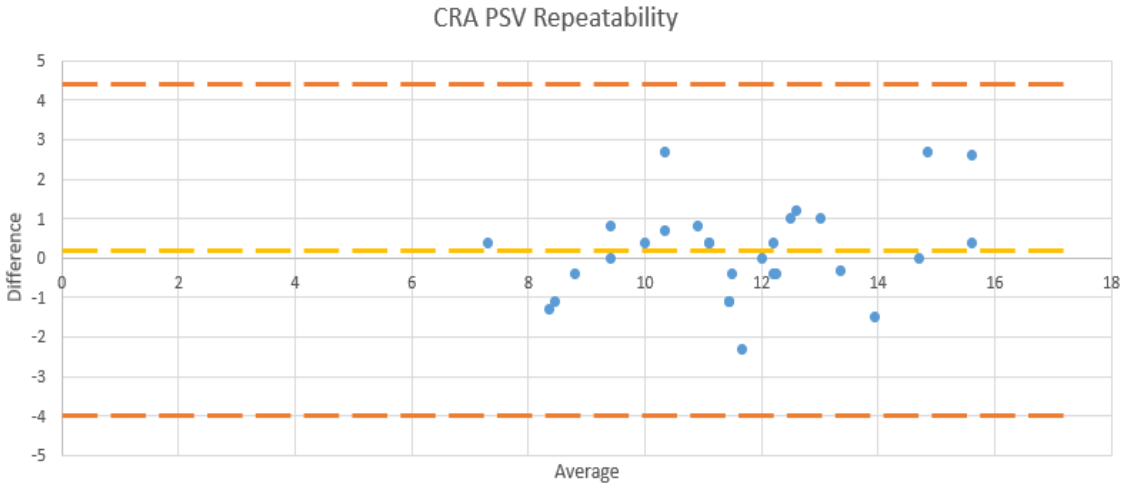

Figure 7. Bland-Altman plot showing good repeatability of CRA PSV measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.77 (pre-treatment ave ± SD: 11.35 ± 3.17cm/s; baseline: 11.54 ± 2.40cm/s). Therefore, when patients came one week apart without major changes to their diet, the CRA PSV remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.11 (baseline ave ± SD: 11.54 ± 2.40cm/s; post-treatment: 12.32 ± 2.61cm/s). Therefore, the CRA PSV did not significantly increase

after one month of 240mg/day ginkgo supplementation, as depicted by the yellow line on Figure 8.

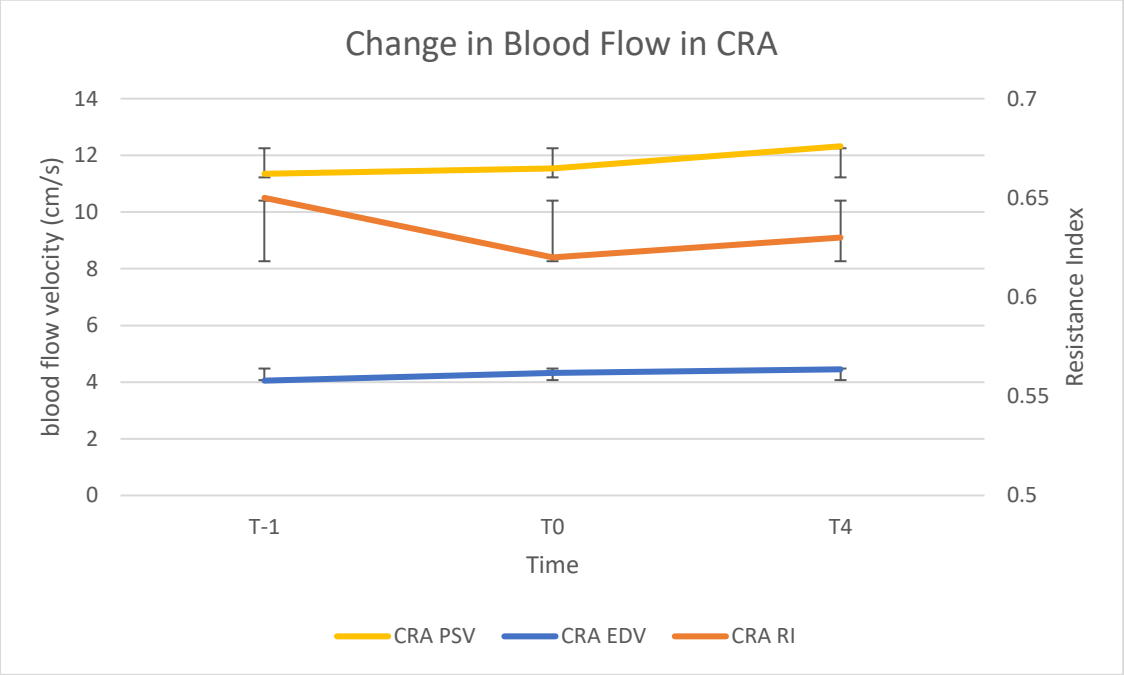

Figure 8. Change in three blood flow parameters in the central retinal artery. There are no significant increases in any blood flow parameter.

There was no correlation between CRA PSV and axial length. The Pearson correlation coefficient for axial length and velocity change R-value was 0.12. Therefore only 12% of the PSV increase could be explained by the length of the eyeball. The p-value was 0.69, so this result was not significant.

There was no correlation between CRA PSV and systemic blood pressure. The Pearson correlation coefficient for systolic BP and velocity change R-value was 0.05. Therefore only 5% of the PSV increase could be explained by the systolic BP. The p-value was 0.87, so this result was not significant. The Pearson correlation coefficient for

diastolic BP and velocity change R-value was 0.01. Therefore only 1% of the PSV increase could be explained by the diastolic BP. The p-value was 0.98, so this result was not significant.

There was no correlation between CRA PSV and OPP. The Pearson correlation coefficient for OPP and velocity change R-value was 0.10. Therefore only 10% of the PSV increase could be explained by the OPP. The p-value was 0.75, so this result was not significant.

Central Retinal Artery

End Diastolic Velocity

The data for CRA EDV was normally distributed with a p-value of 0.96. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 9. Most differences between measurements were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

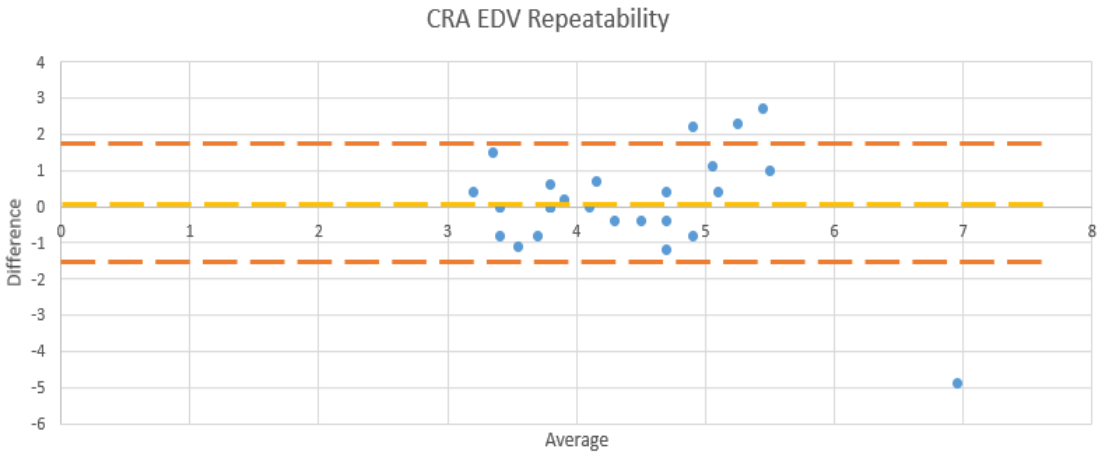

Figure 9. Bland-Altman plot showing good repeatability of CRA EDV measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.50 (pre-treatment ave ± SD: 4.05 ± 2.04cm/s; baseline: 4.32 ± 1.03cm/s). Therefore, when patients came one week apart without major changes to their diet, the CRA EDV remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.32 (baseline ave ± SD: 4.32 ± 1.03cm/s; post-treatment: 4.45 ± 1.11cm/s). Therefore, the CRA EDV did not significantly increase after one month of 240mg/day ginkgo supplementation, as depicted by the blue line in Figure 8.

There was no correlation between CRA EDV and axial length. The Pearson correlation coefficient for axial length and velocity change R-value was 0.07. Therefore only 7% of the EDV increase could be explained by the length of the eyeball. The p-value was 0.82, so this result was not significant.

There was no correlation between CRA EDV and systemic blood pressure. The Pearson correlation coefficient for systolic BP and velocity change R-value was 0.12. Therefore only 12% of the EDV increase could be explained by the systolic BP. The p-value was 0.69, so this result was not significant. The Pearson correlation coefficient for diastolic BP and velocity change R-value was 0.35. Therefore only 35% of the EDV increase could be explained by the diastolic BP. The p-value was 0.23, so this result was not significant.

There was no correlation between CRA EDV and OPP. The Pearson correlation coefficient for OPP and velocity change R-value was 0.28. Therefore only 28% of the EDV increase could be explained by the OPP. The p-value was 0.35, so this result was not significant.

Central Retinal Artery

Resistance Index

The data for CRA RI was normally distributed with a p-value of 0.66. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 10. Most differences between measurements in a single visit were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

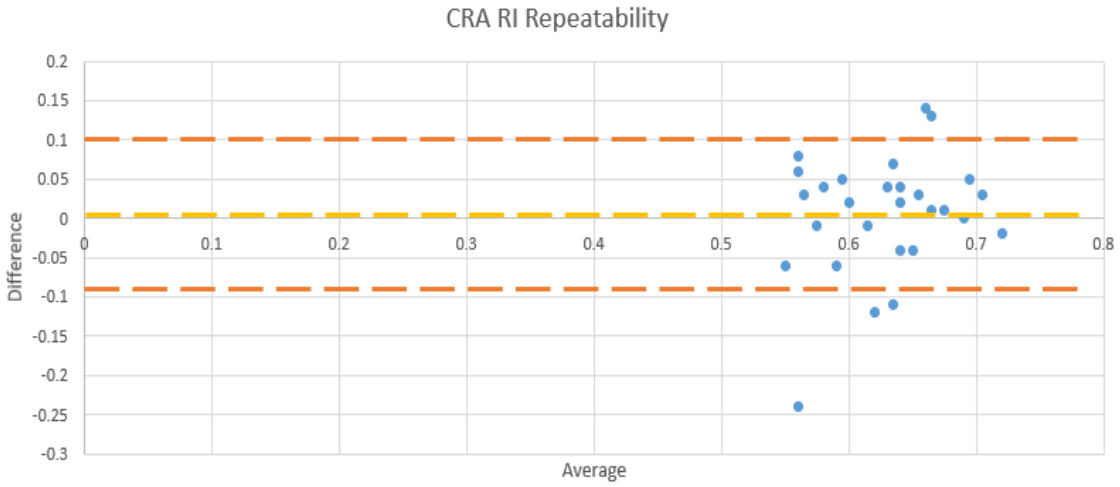

Figure 10. Bland-Altman plot showing good repeatability of CRA RI measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.27 (pre-treatment ave ± SD: 0.65 ± 0.10cm/s; baseline: 0.62 ± 0.05cm/s). Therefore, when patients came one week apart without major changes to their diet, the CRA RI remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.34 (baseline ave ± SD: 0.62 ± 0.05cm/s; post-treatment: 0.63 ± 0.07cm/s). Therefore, the CRA RI did not significantly decrease after one month of 240mg/day ginkgo supplementation, as depicted by the orange line in Figure 8.

There was no correlation between CRA RI and axial length. The Pearson correlation coefficient for axial length and RI change R-value was 0.11. Therefore only 11% of the RI change could be explained by the length of the eyeball. The p-value was 0.72, so this result was not significant.

There was no correlation between CRA RI and systemic blood pressure. The Pearson correlation coefficient for systolic BP and RI change R-value was 0.04. Therefore only 4% of the RI change could be explained by the systolic BP. The p-value was 0.89, so this result was not significant. The Pearson correlation coefficient for diastolic BP and RI change R-value was 0.31. Therefore only 31% of the RI change could be explained by the diastolic BP. The p-value was 0.29, so this result was not significant.

There was no correlation between CRA RI and OPP. The Pearson correlation coefficient for OPP and RI change R-value was 0.17. Therefore only 17% of the RI change could be explained by the OPP. The p-value was 0.59, so this result was not significant.

Short Posterior Ciliary Arteries

Peak Systolic Velocity

The data for SPCA PSV was normally distributed with a p-value of 0.59. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 11. The difference between measurements in a single visit were within ±1.96 standard deviation, indicating excellent repeatability of measurements with low intra-researcher variability.

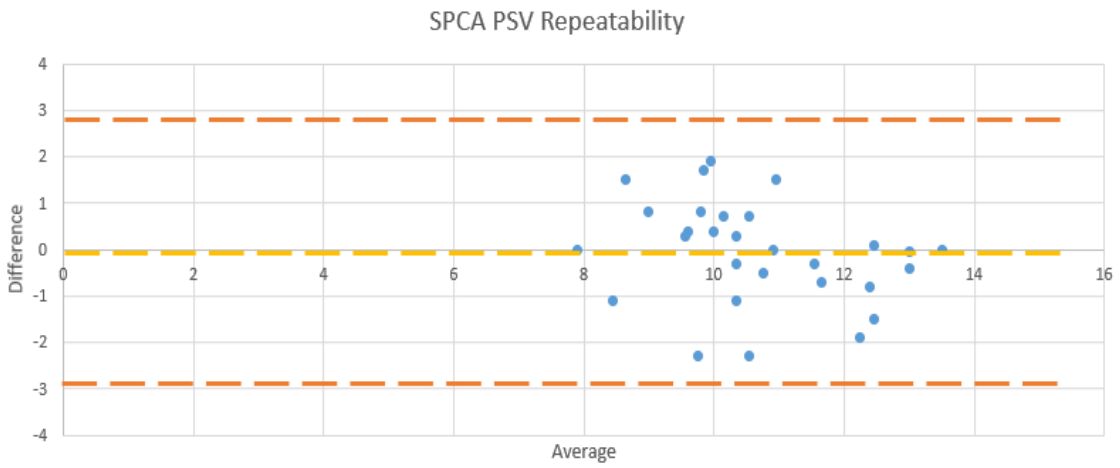

Figure 11. Bland-Altman plot showing excellent repeatability of SPCA PSV measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.34 (pre-treatment ave ± SD: 9.95 ± 2.14cm/s; baseline: 10.56 ± 1.87cm/s). Therefore, when patients came one week apart without major changes to their diet, the SPCA PSV remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.0025 (baseline ave ± SD: 10.56 ± 1.87cm/s; post-treatment: 11.58 ± 1.97cm/s). Therefore, the SPCA PSV significantly increased after one month of 240mg/day ginkgo supplementation, as depicted by the red line in Figure 12. Individual changes in the SPCA PSV of all thirteen subjects from pre-treatment (T_{-1}) to baseline (T_0) to post-treatment (T_4), are shown on Figure 13.

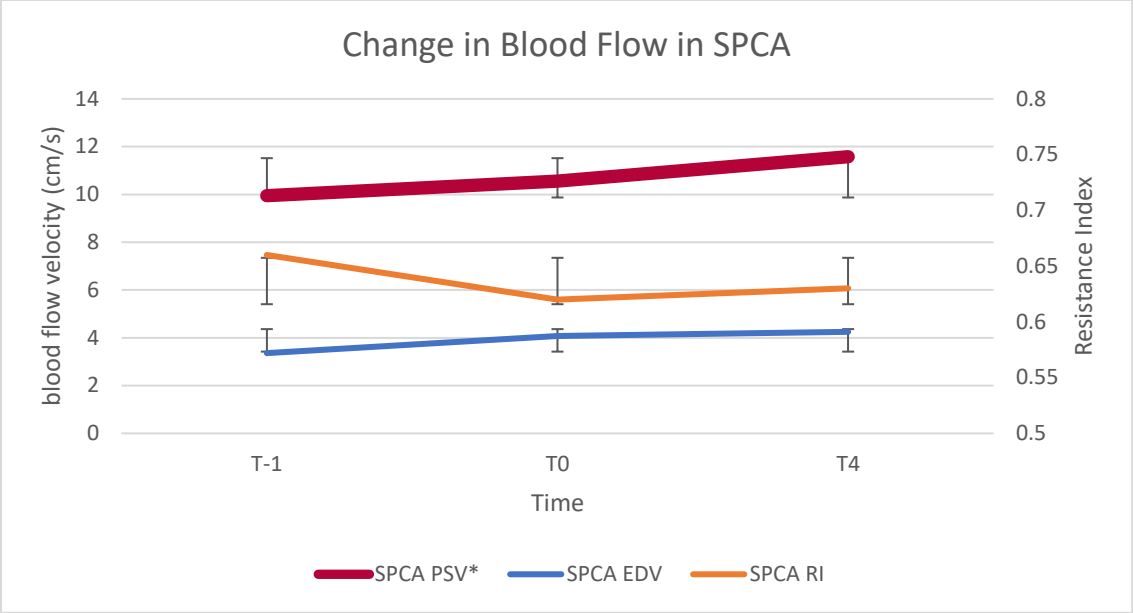

Figure 12. Change in three blood flow parameters in the short posterior ciliary arteries. A significant increase in SPCA PSV is depicted by the red line.

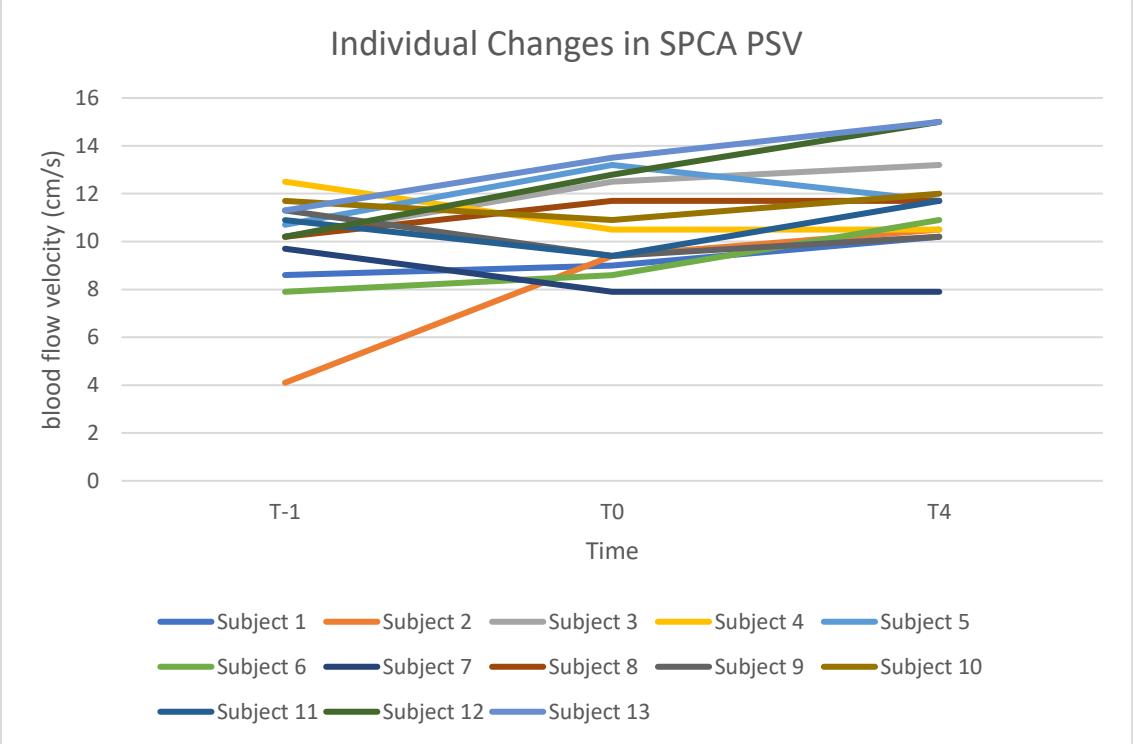

Figure 13. Individual changes in the short posterior ciliary artery's peak systolic velocity for all thirteen subjects.

There was no correlation between SPCA PSV and axial length. The Pearson correlation coefficient for axial length and velocity change R-value was 0.22. Therefore only 22% of the PSV increase could be explained by the length of the eyeball. The p-value was 0.47, so this result was not significant.

There was no correlation between SPCA PSV and systemic blood pressure. The Pearson correlation coefficient for systolic BP and velocity change R-value was 0.28. Therefore only 28% of the PSV increase could be explained by the systolic BP. The p-value was 0.35, so this result was not significant. The Pearson correlation coefficient for diastolic BP and velocity change R-value was 0.36. Therefore only 36% of the PSV increase could be explained by the diastolic BP. The p-value was 0.22, so this result was not significant.

There was no correlation between SPCA PSV and OPP. The Pearson correlation coefficient for OPP and velocity change R-value was 0.29. Therefore only 29% of the PSV increase could be explained by the OPP. The p-value was 0.33, so this result was not significant.

Short Posterior Ciliary Arteries

End Diastolic Velocity

The data for SPCA EDV was normally distributed with a p-value of 0.69. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 14. Most differences between measurements were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

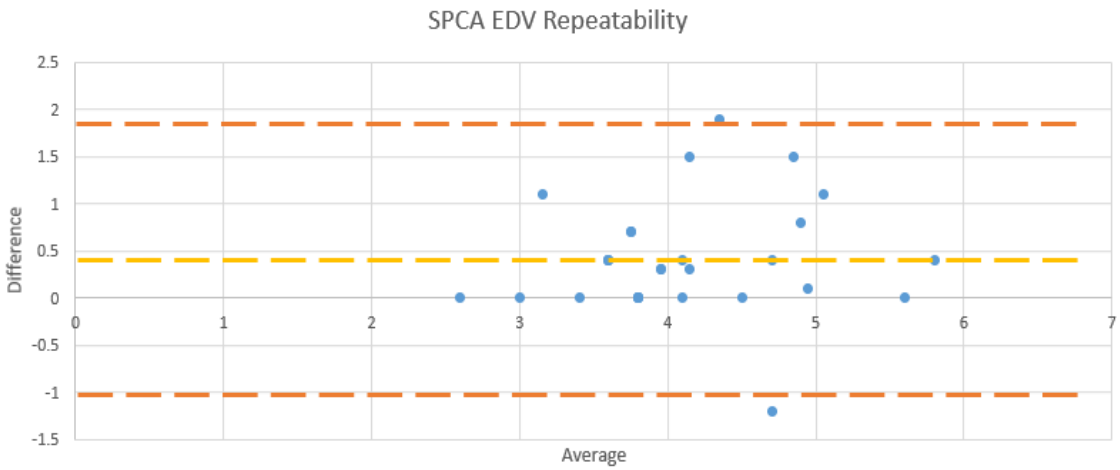

Figure 14. Bland-Altman plot showing good repeatability of SPCA EDV measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.06 (pre-treatment ave ± SD: 3.36 ± 1.38cm/s; baseline: 4.07 ± 1.34cm/s). Therefore, when patients came one week apart without major changes to their diet, the SPCA EDV remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.29 (baseline ave ± SD: 4.07 ± 1.34cm/s; post-treatment: 4.25 ± 1.06cm/s). Therefore, the SPCA EDV did not significantly increase after one month of 240mg/day ginkgo supplementation, as depicted by the blue line in Figure 12.

There was no correlation between SPCA EDV and axial length. The Pearson correlation coefficient for axial length and velocity change R-value was 0.07. Therefore only 7% of the EDV increase could be explained by the length of the eyeball. The p-value was 0.83, so this result was not significant.

There was no correlation between SPCA EDV and systemic blood pressure. The Pearson correlation coefficient for systolic BP and velocity change R-value was 0.08.

Therefore only 8% of the EDV increase could be explained by the systolic BP. The p-value was 0.79, so this result was not significant. The Pearson correlation coefficient for diastolic BP and velocity change R-value was 0.36. Therefore only 36% of the EDV increase could be explained by the diastolic BP. The p-value was 0.23, so this result was not significant.

There was no correlation between SPCA EDV and OPP. The Pearson correlation coefficient for OPP and velocity change R-value was 0.14. Therefore only 14% of the EDV increase could be explained by the OPP. The p-value was 0.65, so this result was not significant.

Short Posterior Ciliary Arteries

Resistance Index

The data for SPCA RI was normally distributed with a p-value of 0.61. Repeatability of measurements at each visit for all thirteen subjects are shown on Figure 15. Most differences between measurements in a single visit were within ±1.96 standard deviation, indicating good repeatability of measurements with low intra-researcher variability.

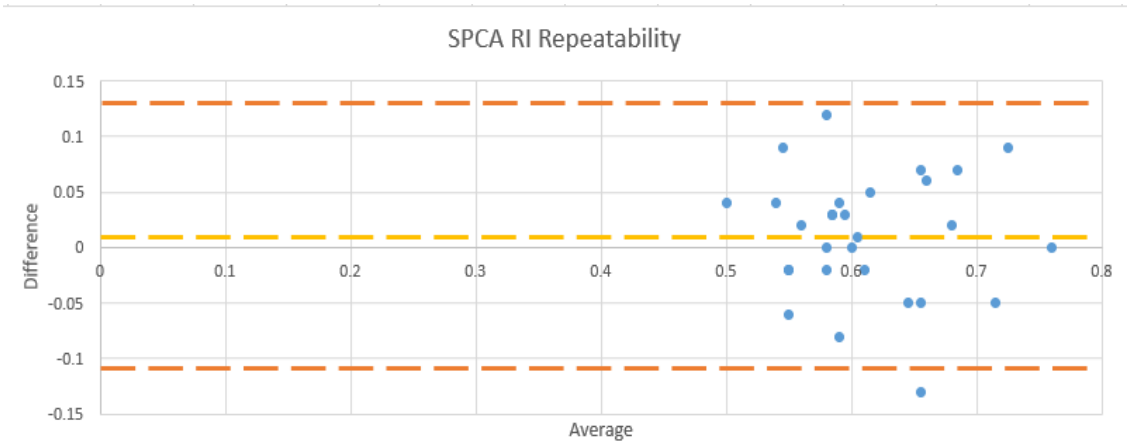

Figure 15. Bland-Altman plot showing good repeatability of SPCA RI measurements.

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the p-value was 0.09 (pre-treatment ave ± SD: 0.65 ± 0.10cm/s; baseline: 0.62 ± 0.05cm/s). Therefore, when patients came one week apart without major changes to their diet, the SPCA RI remained stable. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the p-value was 0.40 (baseline ave ± SD: 0.62 ± 0.05cm/s; post-treatment: 0.63 ± 0.07cm/s). Therefore, the SPCA RI did not significantly decrease after one month of 240mg/day ginkgo supplementation, as depicted by the orange line in Figure 12.

There was no correlation between SPCA RI and axial length. The Pearson correlation coefficient for axial length and RI change R-value was 0.09. Therefore only 9% of the RI change could be explained by the length of the eyeball. The p-value was 0.70, so this result was not significant.

There was no correlation between SPCA RI and systemic blood pressure. The Pearson correlation coefficient for systolic BP and RI change R-value was 0.11. Therefore only 11% of the RI change could be explained by the systolic BP. The p-value was 0.72, so this result was not significant. The Pearson correlation coefficient for

diastolic BP and RI change R-value was 0.37. Therefore only 37% of the RI change could be explained by the diastolic BP. The p-value was 0.21, so this result was not significant.

There was no correlation between SPCA RI and OPP. The Pearson correlation coefficient for OPP and RI change R-value was 0.16. Therefore only 16% of the RI change could be explained by the OPP. The p-value was 0.61, so this result was not significant.

Changes in Systolic BP, Diastolic BP, OPP and Pulse Rate

Changes in Systolic and Diastolic BP

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the systolic BP p-value was 0.42 (pre-treatment ave ± SD: 123.31 ± 9.59mmHg; baseline: 125.08 ± 10.87mmHg) and the diastolic BP p-value was 0.45 (pre-treatment ave ± SD: 80.38 ± 8.94mmHg; baseline: 79.23 ± 8.17mmHg). Therefore, the systolic and diastolic blood pressures remained stable when patients came one week apart without major changes to their diet.

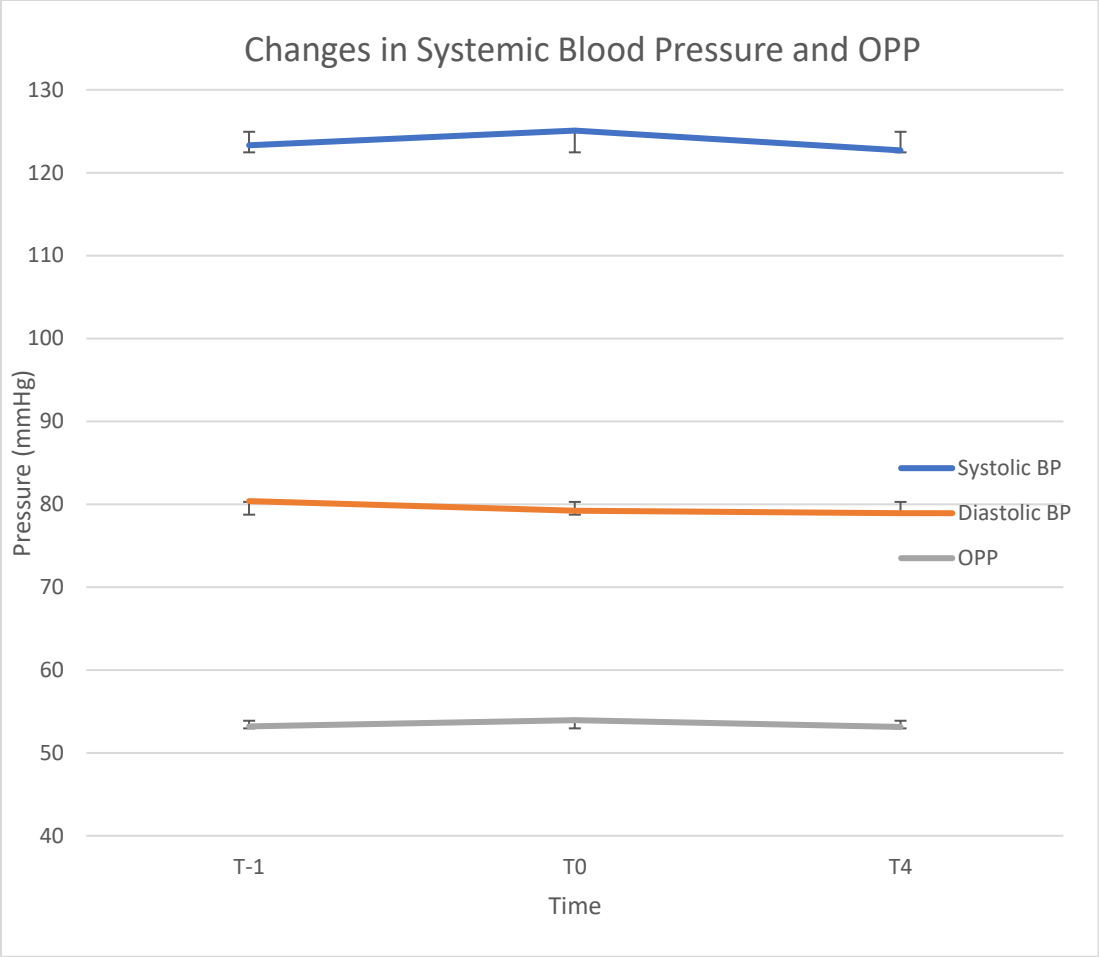

Figure 16. Change in systolic BP, diastolic BP, and OPP over three visits. There are no significant changes in any measurement throughout the study.

Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the systolic p-value was 0.19 (baseline ave ± SD: 125.08 ± 10.87mmHg; post-treatment: 122.69 ± 13.91mmHg) and the diastolic BP p-value was 0.41 (baseline ave ± SD: 79.23 ± 8.17mmHg; post-treatment: 78.92 ± 6.51mmHg). Therefore, as shown in Figure 16, the systolic and diastolic blood pressures did not decrease after one month of 240mg/day ginkgo supplementation.

Changes in OPP

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the OPP p-value was 0.49 (pre-treatment ave ± SD: 53.18 ± 6.38mmHg; baseline: 53.95 ± 6.40mmHg). Therefore, the OPP remained stable when patients came one week apart without major changes to their diet.

Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the OPP p-value was 0.23 (baseline ave ± SD: 53.95 ± 6.40mmHg; post-treatment: 53.13 ± 6.69mmHg). Therefore, as shown in Figure 16, OPP did not increase after one month of 240mg/day ginkgo supplementation.

Changes in Pulse Rate

Comparing the control phase from pre-treatment (T_{-1}) to baseline (T_0), the pulse rate p-value was 0.81 (pre-treatment ave ± SD: 73.62 ± 12.57bpm; baseline: 74.23 ± 12.10bpm). Therefore, the pulse rate remained stable when patients came one week apart without major changes to their diet. Comparing the experimental phase from baseline (T_0) to post-treatment (T_4), the pulse rate p-value was 0.46 (baseline ave ± SD: 74.23 ± 12.10bpm; post-treatment: 73.92 ± 11.37bpm). Therefore, as shown in Figure 17, pulse rate did not change after one month of 240mg/day ginkgo supplementation.

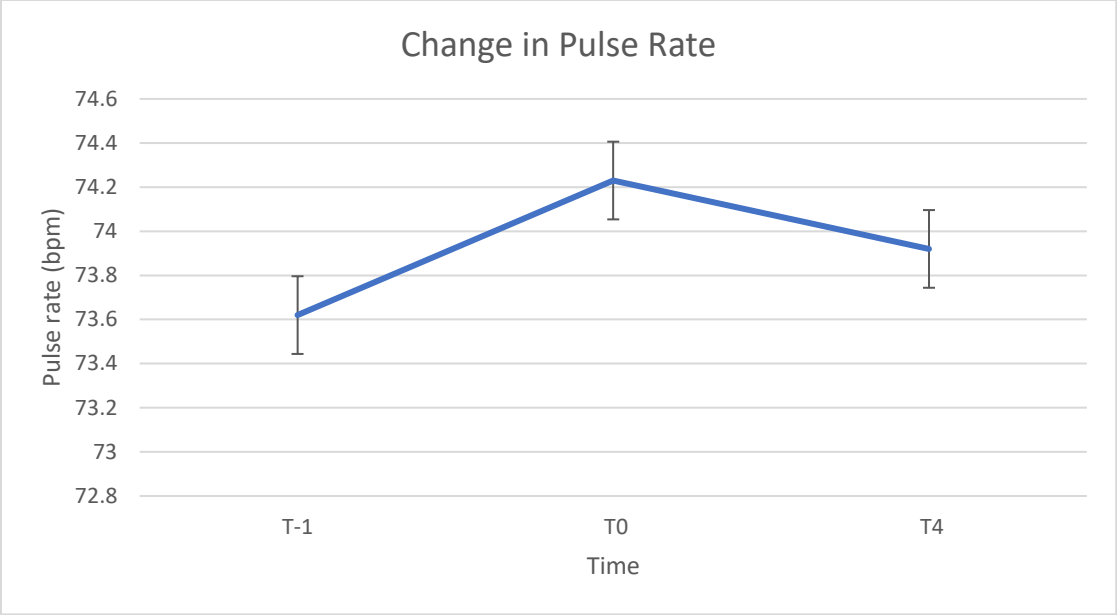

Figure 17. Change in pulse rate over three visits. There is no significant change in pulse rate throughout the study.

Discussion

The outcomes of this study suggest that ginkgo biloba extract (GBE) may increase blood flow in two major retinal ocular arteries. The peak systolic velocity of the ophthalmic artery and short posterior ciliary arteries increased after one month of GBE supplementation (240mg/day). These increases were independent from axial length, systemic blood pressure, and ocular perfusion pressure.

The significance of this work is its novelty as the first prospective, interventional clinical trial in young healthy eyes free from systemic or ocular disease. Demonstrating GBE's ability to increase ocular blood flow in healthy eyes supports the importance it might play as a preventive treatment for eyes with vascular complications. The main strength of the study was the implementation of a 1-week control phase with no GBE supplementation, to control for confounding lifestyle factors such as diet, exercise habits, and sleep patterns that could affect the effect of GBE supplementation during the treatment phase. Other strengths included scheduling visits at the same time during the day to control for the effects of circadian rhythm, and repeating measurements twice to assess repeatability and to minimize intra-observer variability.

The effects of GBE on blood vessel health in animal models and systemic blood flow in humans have been well documented, however the effects on ocular blood flow in human eyes are not yet clear. This study used color doppler imaging to provide insight into the effect of GBE on blood flow following one month of supplementation. A substantial increase in the peak systolic blood velocity in two ocular arteries was identified. In examining the factors contributing to peak systolic velocity, blood flow is known to be directly related to ocular perfusion pressure and inversely related to

resistance.[12] This study found that the ocular perfusion pressure and resistance index of all ocular arteries were stable throughout the course of the study. Thus, there may be an alternative mechanism by which GBE affects blood flow. Further research is needed to elucidate the mechanisms by which GBE acts in human ocular arteries.

The results of this study showing significant increases in the PSV of OA and SPCA should be interpreted with caution. Comparison of the PSV values with the Bland-Altman plots of repeatability reveal that the increases in PSV of OA and SPCA lie within the noise of intra-researcher variability. Table 1 shows that the average OA PSV increased 5.36cm/s from baseline (T_0) to post-treatment (T_4), however Figure 2 shows a large 25cm/s range of measurement repeatability, indicating that the significant OA PSV increase may be within tolerance of intra-researcher variability. Additionally, the individual changes in OA PSV of all thirteen subjects shown in Figure 4 reveals a general trend of slight decrease from pre-treatment (T_{-1}) to baseline (T_0), followed by a slight increase from baseline (T_0) to post-treatment (T_4). The values of PSV at post-treatment (T_4) are comparable to values at pre-treatment (T_{-1}), indicating that the PSV increase may be attributed to normal fluctuation in blood flow. The significant result of SPCA PSV is similar to that of OA PSV. Table 1 shows that the average SPCA PSV increased 1.02cm/s from baseline (T_0) to post-treatment (T_4), however Figure 11 shows a large 6cm/s range of measurement repeatability, indicating that the significant SPCA PSV increase may be within tolerance of intra-researcher variability. Additionally, the individual changes in SPCA PSV of all thirteen subjects shown in Figure 13 reveals a general trend of slight increase from pre-treatment (T_{-1}) to baseline (T_0), followed by another slight increase from baseline (T_0) to post-treatment (T_4). The slight increases of

PSV before and after baseline (T_0) are similar, indicating that the PSV increase may be attributed to normal fluctuation in blood flow. Thus, the significant increases in OA PSV and SPCA PSV found in this study may be partly a result of intra-researcher variability and normal fluctuations in blood flow instead of the effects of GBE.

This study found that GBE did not have a significant effect on the end diastolic velocity. A possible reason for the change in peak systolic blood velocity but not the end diastolic velocity is the nature of the measure. Peak systolic velocity indices are more dependent on peak contractility, whereas end diastolic velocity indices are more related to stroke volume.[13] Given that stroke volume is dependent on both contractility force and the total time span of work, end diastolic velocity is more affected by heart rate. In our study pulse rate was not significantly affected by GBE. As shown in Figure 16, the heart rate of each subject remained stable between baseline visit (T_0) and post-supplement visit (T_4), which could partly explain the lack of significant change of end diastolic velocity in this study. Additionally, echocardiography studies have found that peak systolic velocity is a more sensitive marker to changes in contraction. In one human study evaluating myocardial function of young healthy subjects, inotropic alterations affected peak systolic velocity indices more than end diastolic velocity indices.[14] In another study of left ventricular function in sheep, peak velocity was considerably more affected than the total velocity time integral that is related to end diastolic velocity.[15] Thus, the significant increase in peak systolic velocity but not end diastolic velocity found in this study may be attributed to GBE's influence on blood flow without changes to heart rate, as well as to peak systolic velocity greater sensitivity to change.

The only other study assessing the effects of GBE on ocular blood flow in humans found that daily intake of 120mg over two days significantly increased the ophthalmic artery's end diastolic velocity in glaucoma patients.[6] Their results highlighted the potential of GBE as a treatment for ischemic ocular diseases, which provides another demographic application of GBE compared to this study's population of healthy eyes. Although the mechanisms of glaucomatous damage are not fully known, it is believed that one contributing mechanism is abnormal optic nerve circulation deriving from the short posterior ciliary arteries.[16] In a study evaluating blood flow in the three major retrobulbar vessels in primary open angle glaucoma patients, glaucomatous eyes had significantly decreased peak systolic and end diastolic velocities, and increased resistive index.[17] Specifically, the peak systolic velocities of both the posterior ciliary artery and ophthalmic artery were shown to be further reduced in glaucomatous eyes versus healthy control eyes. Given our finding that GBE significantly increased the PSV in the short posterior ciliary arteries and ophthalmic artery of healthy subjects, there is an opportunity for GBE to have an even greater influence on blood flow velocity in glaucoma patients. Thus, GBE may have a role in both maintenance of good ocular health and treatment of ischemic ocular disease.

Another widespread ocular disorder characterized by changes in vascular supply is myopia. High myopes in particular are at greater risk of developing future pathologies that significantly decrease quality of life, warranting further research on maintaining the health of ocular tissue in myopic eyes. The literature has shown that high myopic eyes have decreased blood flow in the central retinal artery,[18,19] reduced choriocapillary flow,[20] and lower retinal arteriole oxygen saturation.[21–23] Associations have been

established between high myopia and degeneration of ocular tissue caused by reduced ocular blood flow. However, research has not yet been done on the potential beneficial effects of GBE on myopic eyes. Given the described effects of GBE on blood vessel health, GBE may be a safe, low-risk dietary supplement to enhance ocular tissue health in high myopes.

Our significant findings of increased ocular blood flow undermine a potential link between nutrition and eye health. The application of nutrition in ocular health has been extensively studied in age-related macular degeneration (AMD). In a large randomized, double-masked, placebo-controlled trial known as the Age-Related Eye Disease Study (AREDS), 3640 patients with AMD were given daily supplements containing combinations of vitamin C, vitamin E, beta-carotene, zinc, and copper, or a placebo.[24] The study found that daily long-term supplementation with 500mg vitamin C, 400 IU vitamin E, 15mg beta-carotene, 80mg zinc, and 2mg copper reduced the risk of progression from intermediate AMD to late-stage AMD by 25% at 5 years. In a follow-up randomized, double-masked, placebo-controlled clinical study known as AREDS2, the substitution of beta-carotene for the carotenoids lutein and zeaxanthin was also suggested to reduce AMD progression.[25] These carotenoids are naturally found in dark green leafy vegetables as well as various colored fruits,[26] highlighting the importance of a healthy dietary pattern to ocular health. As a result of these trials, the nutritional supplement known as the AREDS formulation as well as recommendations of a diet high in a variety of foods has become a standard of care for patients at risk of developing advanced AMD.

Another application of nutrition in ocular health has focused on dry eye disease. Evidence has shown a correlation between omega-3 fatty acids and treatment of dry eyes.

Omega-3's are termed essential fatty acids because they are unable to be synthesized and must be consumed from diet.[27] Types of omega-3 fatty acids include eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA),[28] which are naturally found in cold water fish and flaxseed oil.[29] In a double blind randomized trial of 264 eyes, patients with dry eyes were given 1000mg daily supplement intake of 325mg EPA and 175mg DHA for 3 months.[30] Results found a significant improvement in subjective symptoms and objective TBUT scores. In another interventional, double-masked study with 105 subjects, the effects of daily supplementation of 1680mg EPA/560mg DHA over 12 weeks was investigated.[31] Statistically significant improvements in tear osmolarity, omega-3 index levels, TBUT, MMP-9, and OSDI symptom scores were noted, undermining the efficacy of nutritional therapy for dry eyes. Dietary intervention of omega-3 fatty acid has shown to be beneficial in improving both subjective and objective measurements of dry eye disease.

The potential therapeutic benefits of dietary supplements as a low-risk, simple, and cost-effective strategy for prevention of ocular disease warrants further investigation. The present study provides new insights into the potential for GBE to increase ocular blood flow in healthy eyes. Several design limitations still existed, including small study size and lack of randomization. The thirteen included subjects were all healthcare workers in the field of optometry, introducing possible bias into the patient population. For future studies, a large-scale randomized double-blind clinical trial in patients with ischemic ocular pathology is necessary to draw stronger conclusions about the effect of GBE.

References

1. Diamond BJ, Shiflett SC, Feiwel N, et al. Ginkgo biloba extract: Mechanisms and clinical indications. *Arch Phys Med Rehabil*. 2000;81(5):668-678.

2. Zhou W, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C. Clinical use and molecular mechanisms of action of extract of Ginkgo biloba leaves in cardiovascular diseases. *Cardiovasc Drug Rev*. 2004;22(4):309-319.

3. Li Y, Zhu X, Wang K, Zhu L, Murray M, Zhou F. Ginkgo biloba extracts (GBE) protect human RPE cells from t-BHP-induced oxidative stress and necrosis by activating the Nrf2-mediated antioxidant defence. *J Pharm Pharmacol*. 2023;75(1):105-116.

4. Delaflotte S, Auguet M, DeFeudis F V., et al. Endothelium-dependent relaxations of rabbit isolated aorta produced by carbachol and by Ginkgo biloba extract. *Biomed Biochim Acta*. 1984;43(8-9):S212-6.

5. Shiyong Y, Yijia X, Peng Z, et al. Ginkgo biloba Extract Inhibits Platelet Activation via Inhibition of Akt. *Integr Med Int*. 2014;1(4):234-242.

6. Chung HS, Harris A, Kristinsson JK, Ciulla TA, Kagemann C, Ritch R. Ginkgo biloba extract increases ocular blood flow velocity. *J Ocul Pharmacol Ther*. 1999;15(3):233-240.

7. Park JW, Kwon HJ, Chung WS, Kim CY, Seong GJ. Short-term effects of Ginkgo biloba extract on peripapillary retinal blood flow in normal tension glaucoma. *Korean J Ophthalmol*. 2011;25(5):323-328.

8. Harris A, Gross J, Moore N, et al. The effects of antioxidants on ocular blood flow in patients with glaucoma. *Acta Ophthalmol*. 2018;96(2):e237-e241.

9. Xie Z, Wu X, Gong Y, Song Y, Qiu Q, Li C. Intraperitoneal Injection of *Ginkgo biloba* Extract Enhances Antioxidation Ability of Retina and Protects Photoreceptors After Light-Induced Retinal Damage in Rats. *Curr Eye Res*. 2007;32(5):471-479.

10. Ranchon I, Gorrand JM, Cluzel J, Droy-Lefaix MT DM. Functional protection of photoreceptors from light-induced damage by dimethylthiourea and Ginkgo biloba extract. *Investig Ophthalmol Vis Sci*. 1999;40(6):1191-1199.

11. Bucolo C, Marrazzo G, Platania CBM, Drago F, Leggio GM SS. Fortified Extract of Red Berry, Ginkgo biloba, and White Willow Bark in Experimental Early Diabetic Retinopathy. *J Diabetes Res*. 2013;2013:432695.

12. van Keer K, Breda JB, Pinto LA, Stalmans I, Vandewalle E. Estimating Mean Ocular Perfusion Pressure Using Mean Arterial Pressure and Intraocular Pressure. *Invest Ophthalmol Vis Sci*. 2016;57(4):2260-2260.

13. Chirinos JA, Segers P, Gupta AK, et al. Time-varying myocardial stress and systolic pressure-stress relationship: Role in myocardial-arterial coupling in hypertension. *Circulation*. 2009;119(21):2798-2807.

14. Thorstensen A, Dalen H, Amundsen BH, Støylen A. Peak systolic velocity indices are more sensitive than end-systolic indices in detecting contraction changes assessed by echocardiography in young healthy humans. *Eur J Echocardiogr*. 2011;12(12):924-930.

15. Bauer F, Jones M, Shiota T, et al. Left ventricular outflow tract mean systolic acceleration as a surrogatefor the slope of the left ventricular end-systolic pressure-volume relationship. *J Am Coll Cardiol*. 2002;40(7):1320-1327.

16. Rojanapongpun P, Drance SM, Morrison BJ. Ophthalmic artery flow velocity in glaucomatous and normal subjects. *BritishJournal ofOphthalmology*. 1993;77:25-29.

17. Birinci Murat Danacı HI, Öge Dilek Erkan hsan N, Birinci H. Ocular Blood Flow in Healthy and Primary Open-Angle Glaucomatous Eyes. *Ophthalmologica*. 2002;216:434-437.

18. Benavente-Pérez A, Hosking SL, Logan NS, Broadway DC. Ocular blood flow measurements in healthy human myopic eyes. *Graefe's Arch Clin Exp Ophthalmol*. 2010;248(11):1587-1594.

19. Shimada N, Ohno-Matsui K, Harino S, et al. Reduction of retinal blood flow in high myopia. *Graefe's Arch Clin Exp Ophthalmol*. 2004;242(4):284-288.

20. Mo J, Duan A, Chan S, Wang X, Wei W. Vascular flow density in pathological myopia: An optical coherence tomography angiography study. *BMJ Open*. 2017;7(2):e013571.

21. Zheng Q, Zong Y, Li L, et al. Retinal vessel oxygen saturation and vessel diameter in high myopia. *Ophthalmic Physiol Opt*. 2015;35(5):562-569.

22. Lim LS, Lim XH, Tan L. Retinal vascular oxygen saturation and its variation with refractive error and axial length. *Transl Vis Sci Technol*. 2019;8(4):22-22.

23. Liu X, He X, Yin Y, et al. Retinal oxygen saturation in 1461 healthy children aged 7–19 and its associated factors. *Acta Ophthalmol*. 2019;97(3):287-295.

24. Evans JR, Lawrenson JG. Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. *Cochrane Database Syst Rev*. 2017;2017(7).

25. Lim MCC, Hoh ST, Foster PJ, et al. Use of optical coherence tomography to assess variations in macular retinal thickness in myopia. *Investig Ophthalmol Vis Sci*. 2005;46(3):974-978.

26. Eisenhauer B, Natoli S, Liew G, Flood VM. Lutein and Zeaxanthin—Food Sources, Bioavailability and Dietary Variety in Age-Related Macular Degeneration Protection. *Nutr 2017, Vol 9, Page 120*. 2017;9(2):120.

27. Chong EWT, Kreis AJ, Wong TY, Simpson JA, Guymer RH. Dietary ω-3 Fatty Acid and Fish Intake in the Primary Prevention of Age-Related Macular Degeneration: A Systematic Review and Meta-analysis. *Arch Ophthalmol*. 2008;126(6):826-833.

28. Rand AL, Asbell PA. Current Opinion in Ophthalmology Nutritional Supplements for Dry Eye Syndrome. *Curr Opin Ophthalmol*. 2011;22(4):279.

29. Kris-Etherton PM, Taylor DS, Yu-Poth S, et al. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr*. 2000;71(1):179S-188S.

30. Bhargava R, Kumar P, Kumar M, Mehra N, Mishra A. A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. *Int J Ophthalmol*. 2013;6(6):811.

31. Epitropoulos AT, Donnenfeld ED, Shah ZA, et al. Effect of Oral Re-esterified Omega-3 Nutritional Supplementation on Dry Eyes. *Cornea*. 2016;35(9):1185.