Diplomarbeit

Choroidal Volume Measurements with Optical Coherence Tomography Before and After External Counterpulsation

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unter der Anleitung von

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Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, 18.04.2021 Jakob Daniel Gran eh.

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Inhaltsverzeichnis

Abkürzungen

ECP: External Counterpulsation
PSRT: Personalized Shear Rate Therapy
EECP: Enhanced External Counterpulsation (trademark by VasoMedical, Inc.)
RAST: Retinal vessel Analysis after personalized Shear rate Therapy
CAD: Coronary Artery Disease
CHF: Congestive Heart Failure
RPE: Retinal Pigment Epithelium
LPCA: Long Posterior Ciliary Arteries
SPCA: Short Posterior Ciliary Arteries
PCA: Posterior Ciliary Arteries
ACA: Anterior Ciliary Arteries
EDI SD-OCT: Enhanced Depth Imaging Spectral-Domain Optical Coherence Tomography
AMD: Age-Related Macular Degeneration
CSR: Central Serous Chorioretinopathy
PCV: Polypoidal Choroidal Vasculopathy
VKH: Vogt-Koyanagi-Harnada Disease
RAO: Retinal Artery Occlusion
CRAO: Central Retinal Artery Occlusion
BRAO: Branch Retinal Artery Occlusion
CLRAO: Cilio-Retinal Artery Occlusion
T2DM: Type 2 Diabetes Mellitus
NO: Nitric Oxide
IOP: Applanation Tonometry
ILM: Internal Limiting Membrane
BM: Basement Membrane
RPE: Retinal Pigment Epithelium
OCTA: Optical Coherence Tomography Angiography
ETDRS: Early Treatment Of Diabetic Retinopathy Study
ECG: Electrocardiography

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Zusammenfassung

Die Arbeit mit dem Titel „Messung des Aderhautvolumens mit optischer Kohärenztomographie vor und nach externer Gegenpulsation“ geht der Frage nach, ob externe Gegenpulsation, eine nicht-invasive Therapie bei koronarer Herzkrankheit, unmittelbare Veränderungen des Aderhautvolumens nach sich zieht. Die Probanden/Probandinnen wurden in drei Gruppen (nichtdiabetische Raucher/Raucherinnen, nichtrauchende Diabetiker/Diabetikerinnen und nichtrauchende Gesunde) eingeteilt. Jeweils unmittelbar vor und nach 45-minütiger externer Gegenpulsation wurde bei allen Studienteilnehmern/Studienteilnehmerinnen eine optische Kohärenztomografie im Enhanced Depth Imaging-Modus durchgeführt. Die Untersuchungen fanden im Rahmen der RAST-Studie (Retinal vessel Analysis after personalized Shear rate Therapy) statt. Das choroidale Volumen hat bei allen Probanden/Probandinnen im Mittel um 2,59% zugenommen (± 2,77%, Minimum von -3,09%, Maximum von 10,19%, p < 0,001). Die Untergruppe der Gesunden zeigte eine signifikante mittlere Zunahme des Aderhautvolumens um 3,22% (± 2,46%, Minimum von 0,26%, Maximum von 7,67%, p < 0,001). In der Gruppe der Diabetiker/Diabetikerinnen konnte eine signifikante Zunahme um durchschnittlich 2,70% nachgewiesen werden (± 2,93%, Minimum von 0,28%, Maximum von 10,19%, p 0,012), während in der Gruppe der Raucher/Raucherinnen eine Zunahme um durchschnittlich 1,76% stattgefunden hat (± 2,98%, Minimum von -3,09%, Maximum von 6,16%, p 0,055). Die subfoveale choroidale Dicke hat in der gesamten Studienpopulation im Schnitt um 4,26% zugenommen (± 6,36%, Minimum von -9,69%, Maximum von 18,21%, p < 0,001). In der gesunden Probandengruppe/Probandinnengruppe hat die subfoveale choroidale Dicke um durchschnittlich 4,41% zugenommen (± 6,58%, Minimum von -9,69%, Maximum von 14,29%, p 0,009), während diese Zunahme in der Gruppe der Raucher/Raucherinnen im Mittel 4,8% betragen hat (± 6,17%, Minimum -2,39%, Maximum 18,21%, p 0,0055) und 3,15% in der Subpopulation der Diabetiker/Diabetikerinnen (± 6,73%, Minimum von -7,31%, Maximum von 14,66%, p 0,152). Die Ergebnisse dieser Studie lassen vermuten, dass externe Gegenpulsation in nichtrauchenden, gesunden Personen sowie jungen Diabetikern/Diabetikerinnen zu einer unmittelbaren Zunahme des Aderhautvolumens führen kann.

Abstract

External counterpulsation is a noninvasive therapy for coronary artery disease shown to improve peripheral flow-mediated dilation.

The purpose of this thesis is to examine potential changes in choroidal volume following external counterpulsation.

Three groups of study participants (healthy, smokers and diabetics) were examined with Enhanced Depth Imaging Spectral Domain Optical Coherence Tomography before and after a 45-minute session of External Counterpulsation. These investigations were part of a larger study focusing on retinal vessel analysis after personalized shear rate therapy (RAST).

Choroidal volume across all groups increased significantly at an average of 2,59% ± 2,77% (range -3,09% to 10,19%, $p < 0,001$). The healthy subgroup showed a significant average augmentation of 3,22% ± 2,46% (range 0,26% to 7,67%; $p < 0,001$). The diabetics showed an average augmentation of 2,70% ± 2,93% (range 0,28% to 10,19%, $p = 0,012$), while smoking study participants showed an average increase in choroidal volume of 1,76% ± 2,98% (range -3,09% to 6,16%, $p = 0,055$). Subfoveal choroidal thickness increased significantly at an average of 4,26% ± 6,36% (range -9,69% to 18,21%, $p < 0,001$) across all groups. Subfoveal choroidal thickness increased at an average of 4,41% ± 6,58% (range -9,69% to 14,29%, p 0,009) in the healthy subgroup, 4,8% ± 6,17% (range -2,39% to 18,21%, p 0,0055) in the smoking group and 3,15% ± 6,73% (range -7,31% to 14,66%, p 0,152) in the diabetic group.

The results of this study suggest that external counterpulsation may in the short term increase choroidal volume in non-smoking, otherwise healthy individuals and early diabetics.

1 Introduction

1.1 Ocular circulation

In ocular circulation a distinction has to be made between retinal and choroidal arterial blood supply.[1,2] While retinal blood flow is autoregulated and hardly affected by the sympathetic nervous system and a large number of drugs, choroidal vessels on the opposite generally respond significantly to the application of vasoactive medication and the influence of sympathetic nervous stimulation.[1] When it comes to the anatomy of the ocular circulation, all of the arterial input originates from the ophthalmic artery, emerging from the internal carotid artery.[2] However, there are fundamental differences when it comes to the vessels anatomical pathways.[2] The long posterior ciliary arteries (LPCA) as well as the short posterior ciliary arteries (SPCA), branching off from the posterior ciliary artery (PCA), perforate the sclera in vicinity to the optic nerve, supplying most of the eye's uveal layer.[2] The PCA itself branches off from the ophthalmic artery.[2] In approximately 20% of the population a cilio-retinal artery arises from the SPCA supplying the retina between the macula and the optic nerve.[2] The SPCA delivers blood to the choroid while the LPCA runs along the inner surface of the sclera supplying the ciliary body and iris through formation of the circulus arteriosus major, an anastomosis with the anterior ciliary arteries (ACA), which derives from the muscular branches of the ophthalmic artery.[3] The retina, on the other hand, is supplied by the central retinal artery, also emerging from the ophthalmic artery, running in the dura mater inferior to the optic nerve, subsequently piercing it and penetrating the sclera at the center of the optic nerve as it splits into a superior and an inferior branch to supply the inner layers of the retina.[2] The macula, an oval-shaped pigmented area with its central pit of densely packed cones, the fovea centralis, being responsible for central, high-resolution, color vision, is exclusively dependent on choroidal circulation.[2] This anatomical peculiarity leads to the phenomenon of the so called cherry-red-spot, a small circular choroid shape that can be seen in patients with central retinal artery occlusion (CRAO) due to the macula's relative translucency and surrounding swelling of infarcted retinal nerve fibres.[22]

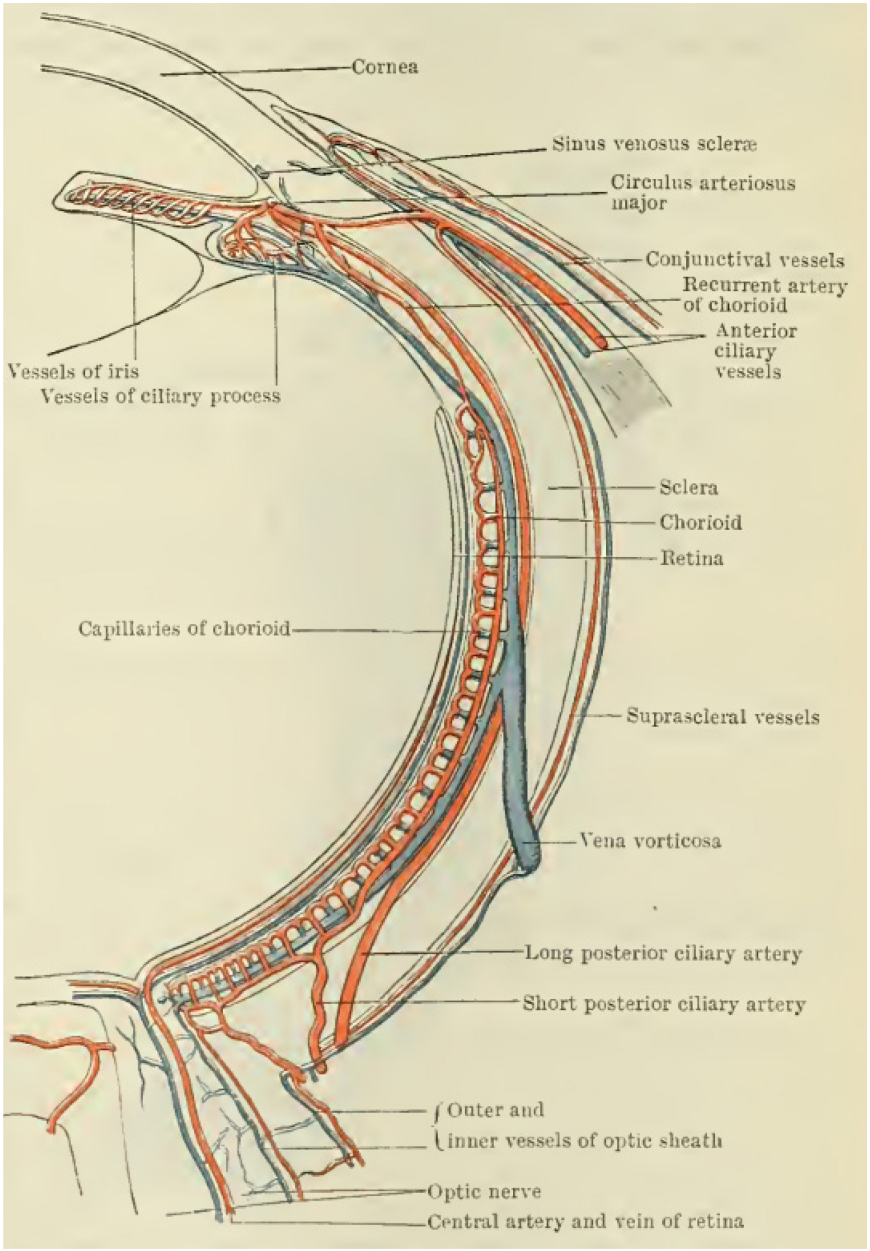


Figure 1 *The ocular circulation. The short posterior ciliary artery can be seen supplying the choroid. (1. Arthur Robinson et al. Cunningham's Text-Book of Anatomy. 1914;(4);p811.) public domain*

1.1.1 Choroid

The choroid is a highly vascularized layer of the eye that, besides numerous other functions, supplies oxygen and nutrients to the retinal pigment epithelium (RPE) and the photoreceptors, removes metabolic waste from phototransduction and probably also maintains a stable temperature in the outer retina.[4] Combined with the iris and ciliary body it forms the uveal tract, also referred to as the vascular layer of the eye.[3] The choroid is made up of four layers with haller's layer being the outermost consisting of larger diameter blood vessels, sattler's layer containing medium diameter vessels, the choroidocapillary layer, made up of capillaries and the innermost limiting layer being bruch's membrane. The choroid is thickest at the posterior pole of the eye.[5] A number of individual factors, such as axial length, age, intraocular pressure, perfusion pressure and endogenous nitric oxide production alongside the vasoactive secretory production of choroidal ganglion cells, endogenous circulating catecholamines, diurnal variation and the choroid’s intrinsic vasomotricity have to be taken into account when determining choroidal thickness.[6]

1.2 Objective

The objective of this thesis is to evaluate the influence of personalized ECP on the choroidal volume in healthy individuals, otherwise healthy type-1 diabetics and smokers. The experiment is the extension of a larger project conducted by the Department of Ophthalmology and the Department of Cardiology at the University Clinic Graz titled "Retinal Vessel Analysis after personalized Shear Rate Therapy (RAST study)" investigating the short-term effects of personalized shear rate therapy on different parameters of ocular circulation.

1.3 Optical coherence tomography

Optical coherence tomography (OCT) is a medical imaging technique used to provide detailed, cross-sectional, in vivo visualization of biological tissue without the need for ionizing radiation.[7] It was introduced for ophthalmic use by researchers of the Massachusetts Institute of Technology in the early 1990s.[8]

1.3.1 Physical background

In OCT, coherent, typically near-infrared light of long wavelengths hits optical scattering media.[9] Subsequently, through low-coherence interferometry, the reflecting sample beam and a reference beam are being superimposed upon one another, creating an interference pattern because of their phase and amplitude differences.[10] The combination with a scanning laser ophthalmoscope allows for precise depth focus reducing much of the background signal which results from reflection out of the imaging plain. Photons that have scattered multiple times can, however, cause artifacts, but only do so with the, relatively small, portion of light that finds its way through the scanning laser ophthalmoscope. Together this results in imaging with micrometer axial resolution.[10] OCT is often referred to as „optical ultrasound“, since it`s mechanics are based on reflections from within tissue to provide cross-sectional images.[10]

1.3.2 Applications in ophthalmology

In ophthalmology and optometry, OCT is primarily used to obtain high-resolution imaging of the retina and anterior segment.[11] Furthermore, the advent of spectral-domain optical coherence tomography (SD-OCT) has enabled clinicians to measure choroidal volume at the eye´s posterior pole by implementing a technique called enhanced depth imaging (EDI). The ability to evaluate choroidal thickness using EDI simplifies diagnostics as well as management of pathologies involving changes in choroidal volume, such as age-related macular degeneration (AMD), central serous chorioretinopathy (CSR), Polypoidal Choroidal Vasculopathy (PCV), Vogt-Koyanagi-Harnada Disease (VKH), white dot syndromes and high myopia.[11–13]

1.4 External counterpulsation

External Counterpulsation is a non-invasive, non-pharmacologic therapeutic approach to refractory angina, heart failure, cardiomyopathy, congestive heart failure (CHF) and hypertension, approved by the United States Food and Drug Administration.[14] In recent years ECP has increasingly drawn the attention of scientific researchers and has been adapted by physicians seeking to aid patients suffering from the above-mentioned conditions, following numerous studies providing encouraging results using ECP.[15]

1.4.1 Method

During ECP, three sets of pneumatic cuffs are placed around the lower body extremities including the buttocks.[14,16] During diastole, these cuffs sequentially contract in order to increase aortic diastolic pressure, leading to augmented coronary blood flow and central venous return, resulting in a plethora of different mechanisms.[14] During conventional ECP, cuff pressures can reach up to 300 mmHg, raising concerns for impaired perfusion of the respective limb.[17]

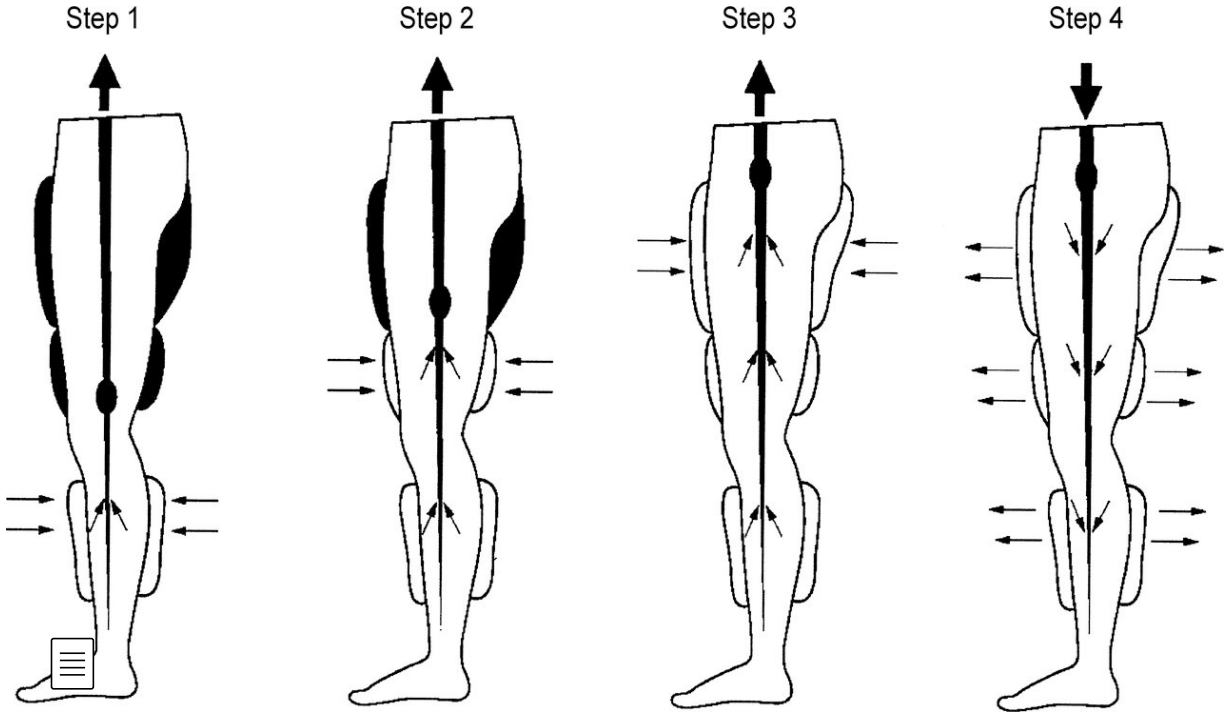


Figure 2. External counterpulsation works through sequential inflation of pneumatic cuffs around the lower extremities, causing retrograde turbulent blood flow and increasing aortic diastolic pressure. (*Manchanda A, Soran O. Enhanced External Counterpulsation and Future Directions. Step Beyond Medical Management for Patients With Angina and Heart Failure. J Am Coll Cardiol. 2007;50(16):1523-1531. doi:10.1016/j.jacc.2007.07.024)*

1.4.2 Physiological Background

ECP leads to immediate improvement in coronary perfusion attributed mainly to increase in diastolic aortic pressure and the effects of elevated plasma levels of the two endothelium-derived vasodilators nitric oxide and prostacyclin as a consequence of increased shear stress.[14,19] Long-term personalized shear rate therapy stimulates the vascular system in a way comparable to physical exercise regarding nitric oxide release.[18] Contraction of the pneumatic cuffs causes retrograde turbulent blood flow in the femoral arteries and aorta (aortic counterpulsation), while the shear stimulus in the brachial arteries is antegrade laminar.[14,19,20] Cardiac workload is reduced by deflation of the cuffs immediately before onset of systole, decreasing vascular resistence.[14] Vascular shear stress also plays an integral role in the presumed long-term therapeutic consequences of ECP by eliciting the activation of a number of different signaling cascades in endothelial cells, such as the upregulation of vascular endothelial growth factors and platelet-derived growth factors, promoting angiogenesis in the form of coronary collateral development and recruitment (flow induced vascular remodeling).[14] Nuclear imaging and positron emission tomography consolidate these effects by showing improved myocardial perfusion following a six week period of ECP therapy.[14]

1.4.3 Therapeutic Applications

A typical treatment regimen of 35 weekly one-hour sessions of ECP has shown to significantly improve the long term quality of life for patients suffering from chronic stable angina, increasing exercise time and reducing the frequency of anginal symptoms.[14] Lawson et al. have demonstrated that improvements in myocardial perfusion in patients with chronic angina pectoris treated with ECP are maintained for at least 3 years post therapy.[21] Furthermore, ECP has been used successfully in patients with coronary artery disease who did not respond to medical or surgical therapy.[22] After controlling for non-responders (missing signs of early improvement in radionuclide stress perfusion imaging), patients with coronary artery disease suffered fewer major adverse cardiovascular events.[22] When it comes to congestive heart failure, levels of brain-natriuretic peptide as well as uric acid significantly reduced in patients undergoing sessions of ECP 5 days a week over a 7-week period while simultaneously increasing left ventricular ejection fraction.[23]

External counterpulsation has further proven its efficacy in the treatment of hypertension. After undergoing personalized shear rate therapy for 30 sessions over the course of 6 weeks (5 times per week), patients demonstrated a decrease in arterial stiffness and lowering of 24-hour blood pressure (systolic by 15 mmHg and diastolic by 8 mmHg).[15] personalized shear rate therapy has also shown promising results for patients with peripheral artery disease, improving endothelial function (determined by flow-mediated endothelium dilation of the brachial artery), plasma nitrate increase and pain-free walking distance following 30 hours of therapy.[17,18]

1.4.4 Other Medical Uses

Off-label applications for non-cardiac conditions include treatment of restless legs syndrome, erectile dysfunction and hepatorenal syndrome along with retinal artery occlusion.[14] A 2004 study conducted by the departments of cardiology and angiology of the Friedrich-Alexander-University in Germany found enhanced external counterpulsation to increase perfusion in ischemic retinal areas immediately after treatment, suggesting enhanced external counterpulsation as a potentially useful and safe procedure in patients with central retinal artery occlusion (CRAO) or branch retinal artery occlusion (BRAO) to accelerate recovery. However, the latter study failed to show a positive impact of enhanced ECP on visual recovery.[24,25] Additionally, a 2005 study examining the effects of enhanced external counterpulsation on patients suffering from carotid artery stenosis in combination with drugs for regulating blood pressure, blood sugar, neurotrophy, anticoagulation and improvement of microcirculation found significant improvement in vision and optical hemodynamics, deeming this treatment method promising in reducing the incidence of ophthalmic and cerebral complications associated with carotid artery stenosis.[26] Recent findings even suggest a benefiting effect of ECP on blood sugar management in patients with Type 2 Diabetes Mellitus (T2DM) by significantly decreasing fasting plasma glucose, HbA1c and insulin resistance.[27] The presumed mechanism of action involves amplification of the nitric oxide (NO)-mediated uptake of glucose into the skeletal muscle as a result of upregulated endogenous NO bioavailability attributed to an increase in shear stress.[27]

1.5 Personalized shear rate therapy

Personalized shear rate therapy is an adaption of external counterpulsation utilizing doppler-ultrasound assisted assessment of the individual intraarterial shear rate in order to facilitate a lowering of treatment pressures to a maximum of 160 mmHg during counterpulsation.[8] First, lower treatment pressures are favorable because it has been shown that therapy pressures exceeding 220 mmHg can decrease blood flow velocity, mitigating the positive effects of external counterpulsation. Secondly, patients suffering from peripheral artery disease may benefit from the advent of personalized shear rate therapy, since this patient population had to be excluded from ECP to date because of the aforementioned risk of decreased blood flow to the endangered peripheral tissue.[17] In figure 5 a subject can be seen undergoing personalized shear rate therapy.

2. Materials and Methods

2.1 Investigational plan

2.1.1 Study design

This study was designed as a non-invasive, interventional, parallel trial.
Informed written consent was obtained from all subjects beforehand. The institutional review board of the Medical University of Graz prospectively approved all study procedures. The final group size was set to 19, accounting for an anticipated 10% dropout rate to ensure statistical significance. One should note that the sample size calculation was performed with the main outcome parameter of the RAST study in consideration; that is flicker response of the retinal vessel diameter. The following 3 groups of participants were predefined: healthy controls, smokers and diabetics.

2.1.2 Study objective

The main objective of the RAST study was to investigate changes in static and dynamic retinal vessel diameter after personalized shear rate therapy. This thesis focuses on the examination of choroidal volume changes before and after external counterpulsation, utilizing the EDI-SD-OCT scans acquired during the RAST study. Furthermore, the researchers conducting the trial aimed to establish a standardized study regimen to be expandable to clinical research in patients with diabetes, arterial hypertension and retinal vascular disorders.

2.1.3 Selection of study population

Three groups of participants were examined, consisting of healthy (in the context of the study meaning the absence of diagnosed diabetes, epilepsy or ocular disease) controls, smokers and diabetics. Each subject was given a specific code for identification consisting of the abbreviation HH (for “Herzhose”) and their respective examination number in chronological order.

Study participants had to meet the following criteria:

Healthy controls:	healthy subjects clear refractive media of at least one eye age over 18 non-smokers non-former-smokers non-diabetics no medication except for contraception
Smokers:	healthy subjects clear refractive media of at least one eye age over 18 smokers
Diabetics:	diabetics (Type 1 or 2) clear refractive media of the eye age over 18 non-smokers non-former-smokers no medication except for anti-diabetic drugs (this includes no blood pressure lowering medication)

2.1.3.1 Baseline parameters

The baseline parameters age, gender, physical activity, refraction, blood pressure, intraocular pressure, smoking pack years, choroidal volume, subfoveal choroidal volume as well as time of OCT examination were measured prior to ECP (shown in Table 1).

2.1.3.1 Exclusion criteria

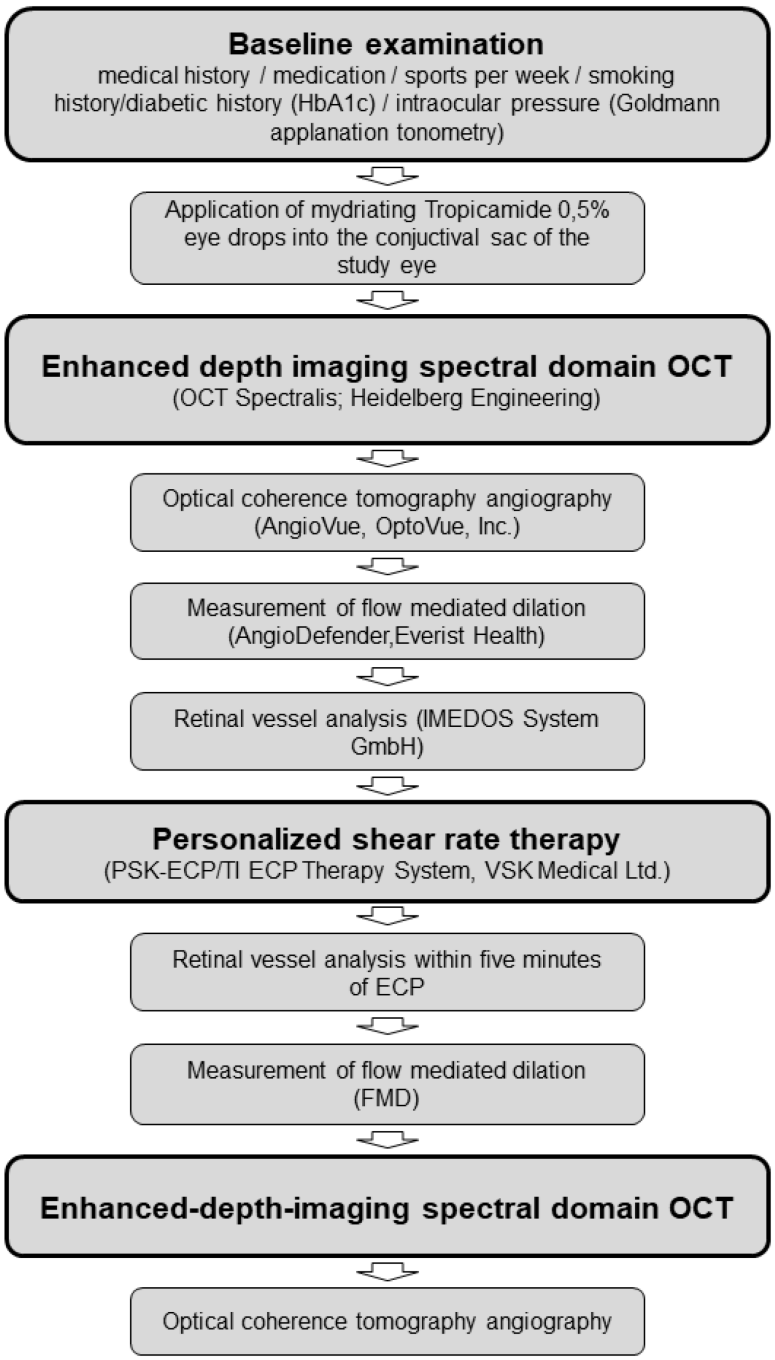
Pregnant women, patients with a history of eye surgery or current treatment with ocular medication as well as subjects suffering from epilepsy or indicating a family history of epilepsy were excluded from the study. Further, anyone presenting with abnormalities preventing the investigator from acquiring reliable measurement data also had to be excluded from the trial.

subject	diabetic (y1,n0)	smoker (y1,n0)	age	gender (m1,f0)	sports per week	refraction of examined eye (dpt)	blood pressure systolic preECP (mmHg)	blood pressure diastolic preECP (mmHg)	intraocular pressure preECP (mmHg)	time of day	pack years	choroidal volume preECP (mm^3)	subfoveal choroidal thickness preECP (µm)
HH01	0	0	32	1	2	0	114	69	12		0		
HH02	0	0	24	1	0	0	110	65	16	14:42	0		357
HH03	0	0	21	0	2	0	127	77	16	15:18	0		362
HH04	0	0	24	1	3	-3,5	130	74	17	14:04	0		
HH05	0	1	20	1	1	-1,5	139	85	17	13:08	2		
HH06	0	0	27	1	0	-5	142	91	16		0		
HH07	0	1	23	0	1	0	108	68	14	15:19	4		390
HH08	1	0	28	0	2	0	115	70	14	15:38	0	7,59	263
HH09	1	0	20	0	2	-3,5	111	65	18	14:10	0	7	266
HH10	0	0	26	0	3	0	120	70	15	10:12	0		423
HH11	0	0	19	1	1	-1	102	61	19	15:14	0	9,65	395
HH12	0	1	24	0	0	0	127	91	16	14:18	9		335
HH13	0	0	25	1	5	0	134	82	18	14:06	0	9,6	330
HH14	0	1	21	1	4	0	128	80	18	15:12	3		341
HH15	0	1	23	1	2	0,5	142	76	16	14:16	3	9,48	352
HH16	0	0	24	1	2	0	120	67	17	09:23	0	8,92	324
HH17	0	0	24	1	2	0	111	69	19	14:15	0	10,6	392
HH18	0	1	35	1	3	-3,25	116	88	17	09:42	5	10,91	414
HH19	1	0	23	1	2	0	108	66	14	13:11	0	8,7	342
HH20	1	0	21	0	2	-0,75	111	70	17	10:18	0	9,33	341
HH21	1	0	21	0	1	1	120	74	18	13:24	0	8,91	369
HH22	1	0	23	0	3	-2	121	75	19	11:03	0	9,34	382
HH23	1	0	24	1	3	-1,5	124	71	16	17:14	0	10,69	353
HH24	1	0	28	1	2	-2	138	85	19	14:13	0	6,14	219
HH25	0	1	29	1	0	0	125	66	17	12:41	7		366
HH26	1	0	25	0	1	-2,5	125	81	16	08:12	0	11,75	483
HH27	0	0	25	1	0	-6	126	72	16	14:29	0	8,14	248
HH28	0	0	20		1	0	117	69	15	18:14	0	8,57	343
HH29	0	0	22	1	7	0	124	64	16	15:16	0	8,78	334
HH30										17:02			
HH31	0	1	29	1	4	0	119	76		17:09	10	11,78	451
HH32	0	1	19	0						13:27		8,74	318
HH33	0	0	23				126	71		16:10		5,82	221
HH34	1	0	22	0	0	-1	114	60	16	16:07	0	8,24	303
HH35	0	1	33	1	3	-1,75	116	65	12	15:35	6	12,33	451
HH36	0	0	19	0	2	0	113	75	16	16:55	0	10	405
HH37	0	0	26	1	4	0	105	65	14	18:50	0	10,09	434
HH38	0	0	23	1	3	-2,5	126	79	13	09:08	0	8,05	328
HH39	0	0	22	0	4	-3,75	98	62	13	13:27	0		197
HH40	0	1	23	1	0	-2,25	109	68	15	14:34	3,5	4,22	133
HH41	0	0	22	0	2	0	110	74	15	13:05	0	5,91	205
HH42	0	0	24	1	1	-2,5	120	75	16	18:26	0	7,32	233
HH43	0	1	26	1	0	0	107	67	11	19:32	3	9,6	318
HH44	0	0	23	1	4	0,5	127	61	14	13:03	0	12,74	494
HH45	0	0	22	1	1	0	124	76	18	18:47	0	7,82	258
HH46	0	1	23	0	2	4,25	96	58	15		4		
HH47	1	0	24	1	4	-0,25	130	73	16		0		
HH48	0	1	26	1	0	0	119	73	16	16:30	6,75	11,55	512
HH49	0	1	19	1	2	-1,5	134	70	14	15:30	3	9,73	373
HH50	1	0	25	1	3	-1,25	132	76	15	17:09	0	7,13	305
HH51	0	1	23	1	1	-1,5	112	69	16	15:05	6	11,14	393
HH52	0	1	25	1	1	-1	113	75	15	14:01	8	9,86	412
HH53	0	1	24	1	3	0	107	72	15	16:20	13,5	10,41	436
HH54	0	1	24	1	2	0	117	70	16	15:48	11	12,74	538

Table 1. *Baseline parameters. OCT image quality was not sufficient for further evaluation in subjects HH01 through HH07, HH10, HH12, HH14, HH25, HH39, HH46 and HH47 while all data for HH30 and some data for HH32 (sports per week, refraction, blood pressure and intraocular pressure) and HH33 (gender, sports per week, refraction, intraocular pressure) were lost. The gender of HH28 was not noted. Missing data fields were left blank.*

2.1.4 Study protocol

Each subject has given written informed consent prior to participating in the examination. Dependencies between subjects and investigators have been ruled out beforehand. For evaluation the investigator chose the eye more suitable. If both eyes were equally suitable, the eye with lower refraction was selected. If equal the right eye was chosen. Measurements and interventions were performed on the study day in the following order:



2.1.5 Outcome variables

The main outcome variable in this thesis is change in choroidal volume after personalized ECP with a planned subgroup analysis between smokers, diabetics and healthy controls. The secondary outcome variable is change in subfoveal choroidal thickness.

2.1.6 Statistical analysis

For determining statistical significance and mean values the t test calculator tool at https://www.graphpad.com/quickcalcs/ttest1/ (23.05.2019), IBM SPSS as well as Microsoft Excel's built in functions were used. Graphs were created using IBM SPSS.

2.2 Materials and Methods of evaluation

2.2.1 Pharmaceuticals

Tropicamide 0.5% (Mydriaticum “Agepha”, Agepha Pharma) was used to dilated the pupil of the subjects. For goldmann applanation tonometry Thilorbin® 4,0 mg/ml (Oxybuprocainhydrochloride) + 0,8 mg/ml (Fluorescein-Sodium) was used.

2.2.2 Enhanced Depth Imaging Spectral Domain OCT (OCT Spectralis, Heidelberg Engineering)

2.2.2.1 Image acquisition

In Enhanced Depth Imaging, the OCT device is placed closer to the eye to obtain a good-quality, inverted image of the choroid by moving the sensitivity curve to the depth of the sclera instead of the vitreoretinal interface.[6,11] Numerous studies have shown high reproducibility of choroidal thickness measurements using EDI.[12] In this research project, certain advantageous characteristics of the Spectralis OCT system (Heidelberg Engineering, Heidelberg, Germany) are being taken advantage of in order to perform EDI as effectively as possible. These advantages include image averaging, eye-tracking (TruTrack), high-speed scanning and low speckle noise. A dense array of 31 EDI-OCT B-scans was recorded prior to external counterpulsation as well as afterwards, spaced 240µm apart from each other while measuring 9.3 mm in lenght, covering a 30 x 25-degree area centered at the fovea resulting in a macular cube.[4,11] At least 25 frames were averaged per scan to improve image quality. The two most peripheral scans at the top and bottom were not manually edited because of their position outside of the EDTRS grid. Figure 2 shows the setup of the OCT measurement. The study participants were asked to focus on a blue target inside the scanning laser ophthalmoscope while resting their chin and forehead inside individually adjusted headgear. On average, this process of image acquisition took about 20 seconds per eye, depending on the participant’s compliance (fixating the blue target, holding both eyes open for a prolonged period of time and holding their head still).

Figure 3. *Spectral domain optical coherence tomography. (University Eye Clinic, Graz, 16.11.2018) The study subject focuses on a blue dot in the device while the images are attained within about 20 seconds.*

2.2.2.2 Determining Choroidal Volume from enhanced depth OCT images

After the image acquisition macular choroidal volume was measured semi-automatically. Essential to this process was the manual segmentation of all B-scans comprising the Early Treatment of Diabetic Retinopathy Study (ETDRS) grid, adding up to 54 B-scan per study participant (27 scans prior to and 27 following external counterpulsation). In the following paragraph this process is described in detail:
First the examiner delineated Bruch's membrane just outside of the retinal pigment epithelium by moving the markers from the inner limiting membrane, where the were originally placed by the devices software.[11] Second the originally devised Bruch's membrane reference line was moved to the chorioscleral junction in a similar fashion. Both step one and two were repeated for all 27 B-scans of the aforementioned cube.[11,32] The resulting volume map between the two reference plains represented the macular choroidal volume and was assessed through use of the automatic retinal thickness/volume map features of the built-in software. Assessment of subfoveal choroidal thickness was performed within the central 1 mm field of the ETDRS grid.

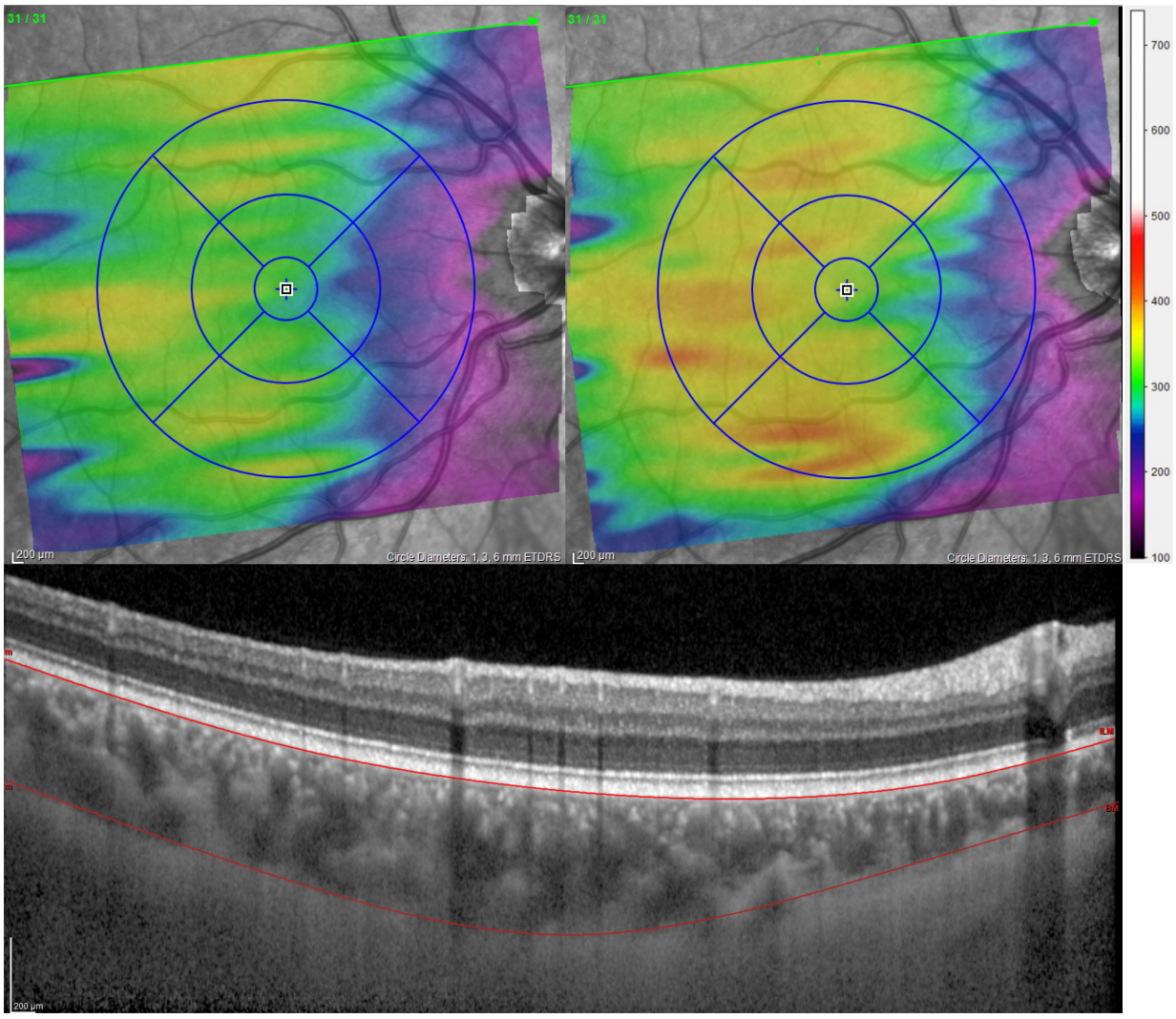


Figure 4. *Changes in choroidal volume can be seen comparing manually segmented OCT scans acquired before (a) and after (b) personalized shear rate therapy (the colour code to the right shows choroidal thickness in µm). Manual segmentation of tissue layers (c) works through moving the red lines representing the internal limiting membrane and Bruch's membrane to comprise the area between Bruch's membrane and the chorioscleral junction, i.e. the choroid layer. (Software: Heidelberg Eye Explorer; Heidelberg Engineering)*

2.2.3 Personalized shear rate therapy (PSK-ECP/TI ECP Therapy System, VSK Medical Ltd.)

This system for external counterpulsation is made up of two pairs of pneumatic cuffs for each of the respective limbs and another cuff for the buttocks, which is strapped around the hips. The patient is connected to the machine through three electrodes for electrocardiography (ECG) as well as through a pulse oximeter. By manually identifying the diastolic interval in the resulting ECG, the investigator precisely determines the point of cuff inflation and deflation. A separate ECG, connected to an ultrasound machine, aids in determining blood flow parameters in the carotid artery during the treatment session. The use of ultrasound is what sets ISRT or personalized shear rate therapy apart from conventional ECP. Monitoring blood flow velocities during the intervention facilitates the application of lower treatment pressures (maxing out at 160 mmHg in ISRT compared to up to 300 mmHg in standard ECP). Starting at manually selected treatment pressures of 40 mmHg, the investigator examines one of the subject's common carotid arteries with doppler-ultrasound and acquires 3 pictures at different pressure levels (40mmHg - 80mmHg – 120 mmHg – 160 mmHg consecutively in the beginning and again at 160 mmHg at the end of the treatment session lasting 45 minutes.

Figure 5. *A test subject undergoing personalized shear rate therapy. (University Eye Clinic, Graz, 16.11.2018)*

3. Results

The OCT scans of 15 healthy subjects, 13 smokers and 11 diabetics qualified for further calculation of choroidal volume.

3.1 Main outcome variable (choroidal volume change)

Choroidal volume across all groups increased at an average of 2,59% ± 2,77% (range -3,09% to 10,19%, p < 0,001). Table 2 shows the mean and median change in choroidal volume by study group. Figure 6 shows the corresponding whisker plots.

3.1.1 Healthy controls

Macular choroidal volume increased in all 15 healthy non-smoking, non-diabetic subjects following external counterpulsation at an average augmentation of 3,22% ± 2,46% (range 0,26% to 7,67%; p < 0,001). This difference was statistically significant. Figure 7 shows the choroidal volume before and after ECP in healthy individuals.

3.1.2 Smokers

Macular choroidal volume increased in 10 out of 13 subjects of the smoking subpopulation following external counterpulsation at an average augmentation of 1,76% ± 2,98% (range -3,09% to 6,16%, p = 0,055). The difference was not statistically significant. Figure 8 shows the choroidal volume before and after ECP in smokers.

3.1.3 Diabetics

Macular choroidal volume increased in all 11 diabetic subjects following external counterpulsation at an average augmentation of 2,70% ± 2,93% (range 0,28% to 10,19%, p = 0,012). Figure 9 shows the choroidal volume before and after ECP in diabetics.

Choroidal Volume Change following ECP

	Mean	Standard Deviation	Median
Choroidal Volume Change across groups	2,59%	2,77%	2,03%
Healthy	3,22%	2,46%	2,87%
Smokers	1,76%	2,98%	1,56%
Diabetics	2,70%	2,93%	2,03%

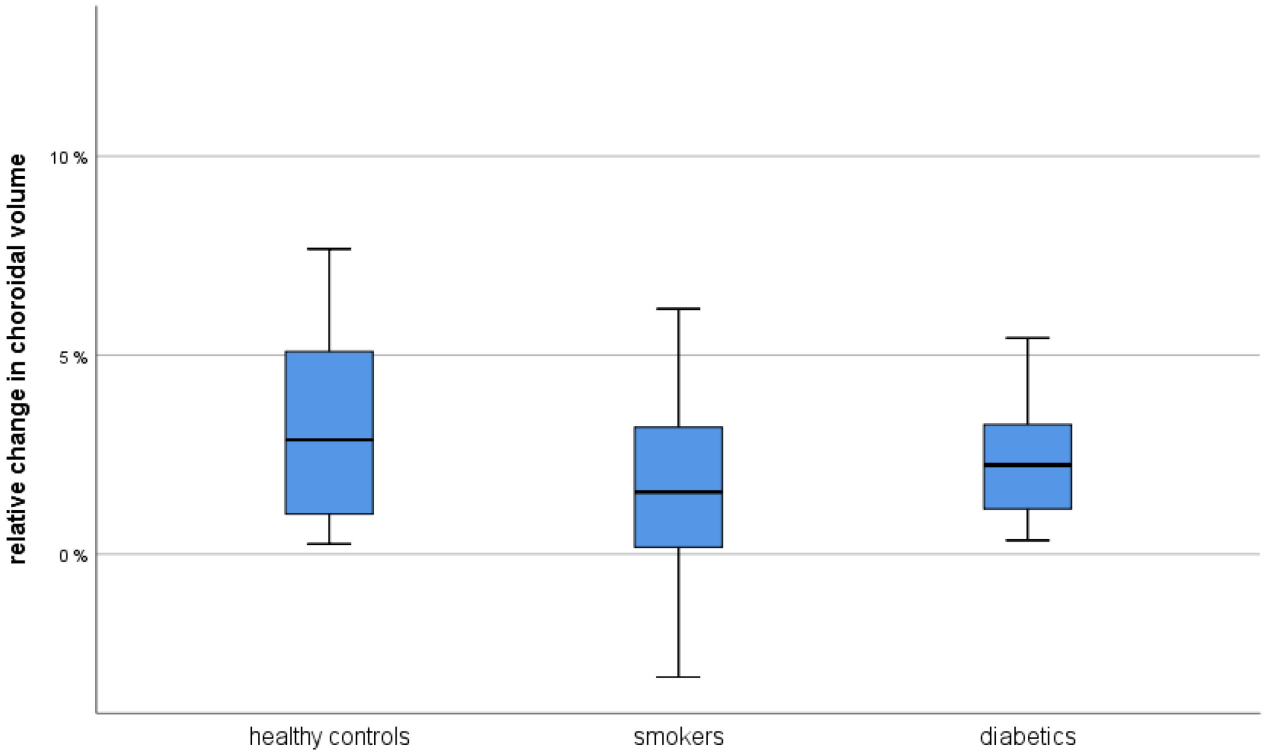


Figure 6. *Relative change in choroidal volume for each subgroup.*

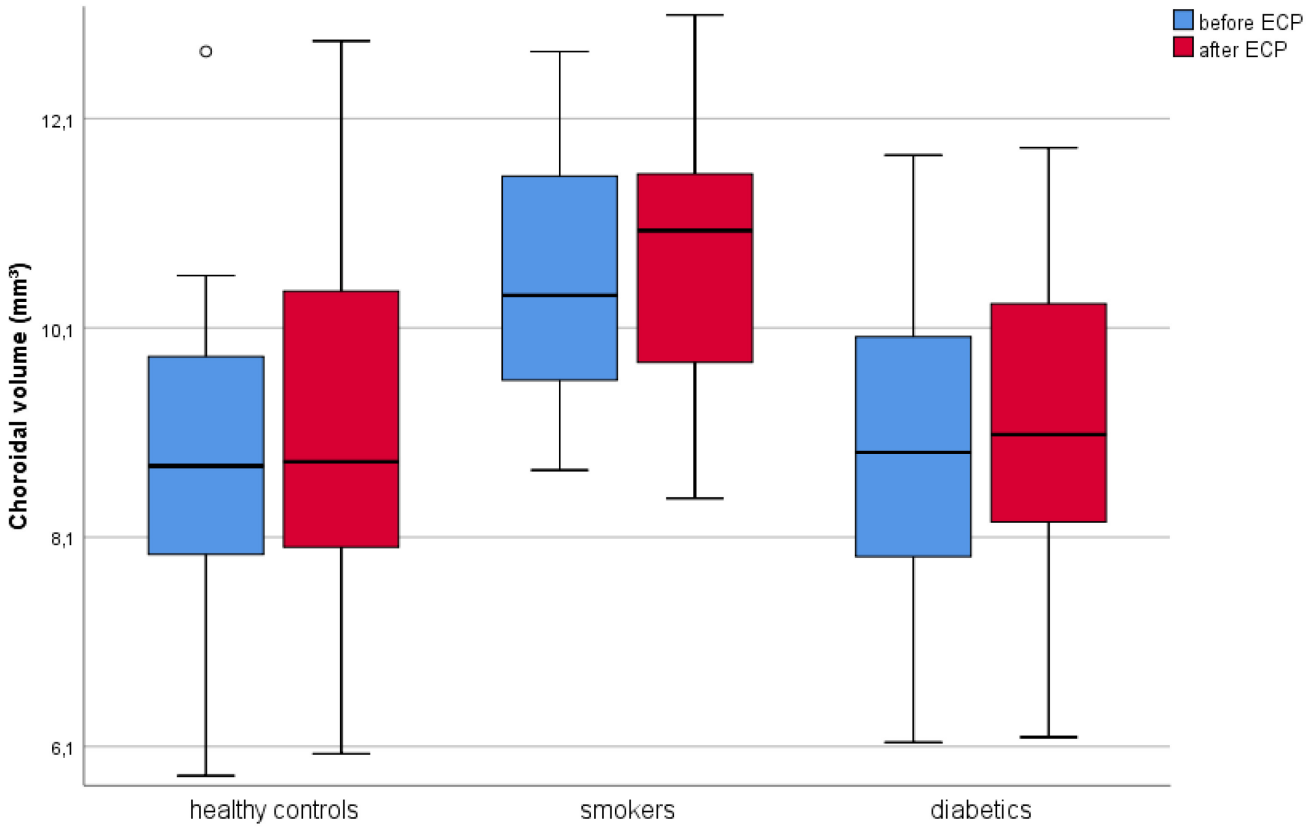


Figure 7*. Absolute change in choroidal volume for each subgroup. The smoking group presented with significantly larger mean choroidal volume than the other two groups.*

3.2 Secondary outcome variable (change in subfoveal choroidal thickness)

Across all groups, subfoveal choroidal thickness significantly increased at an average of 4,26% ± 6,36% (range -9,69% to 18,21%, p < 0,001). Table 3 shows the change in subfoveal choroidal thickness by group.

Subfoveal Choroidal Thickness Change

	Mean	Standard Deviation	Median
Subfoveal Choroidal Thickness Change across groups	4,26%	6,36%	4,11%
Healthy	4,41%	6,58%	4,61%
Smokers	4,80%	6,17%	4,66%
Diabetics	3,15%	6,73%	3,42%

Table 3. *Average change in subfoveal choroidal thickness.*

3.2.1 Healthy

OCT scans of 19 subjects of the healthy subpopulation qualified for evaluation of subfoveal choroidal thickness change. Subfoveal choroidal thickness increased at an average of 4,41% ± 6,58% (range -9,69% to 14,29%, p 0,009) in the healthy control group.

3.2.2 Smokers

Subfoveal choroidal thickness increased at an average of 4,8% ± 6,17% (range -2,39% to 18,21%, p 0,0055) in the smoking group. OCT scans of 17 subjects of the smoking subpopulation qualified for evaluation of subfoveal choroidal thickness change.

3.2.3 Diabetics

Subfoveal choroidal thickness increased at an average of 3,15% ± 6,73% (range -7,31% to 14,66%, p 0,152) in the diabetic group. This difference was not statistically significant. OCT scans of 11 subjects of the diabetic subpopulation qualified for evaluation of subfoveal choroidal thickness change.

4. Discussion

4.1 Interpretation

In this study early diabetics and healthy subjects showed a significant increase in choroidal volume after ECP treatment, while smokers did not. The exact mechanism behind this difference is beyond the scope of this thesis.
ECP gives the chance to have a well standardized setting and less response form smokers is a peculiar finding. The number of pack years did not seem to inversely correlate with the extent of choroidal volume change in the smoking subpopulation. The normal response of our diabetic subpopulation might be attributable to their youth and health. None of them suffered from diabetic retinopathy and their vascular systems may not have differed all that much from those of non-diabetics at the point of examination. The relatively high baseline choroidal volume values throughout the study population (mean 9,21 ± 1,97 mm^3 compared to 7,37 ± 2,18 mm^3 in previous reports by Barteselli et al.), coinciding with the results of Seidel et al. may be explained by the young age of our study participants (mean 24,1 ± 3,3 years), since an inverse correlation between age and choroidal volume has been reported.[4,35]
ECP proposes to simulate physical activity and one can surmise that a vasodilatative effect of ECP and sport on the choroidal vasculature might apply. However, there is conflicting evidence whether physical activity might increase choroidal thickness and thus volume or not, and such an effect might depend on the kind of physical exercise.[36,37,38]

4.2 Confounders, limitations and baseline comparison

It is important to note that this investigation was neither placebo-controlled nor were groups randomized. The researchers were not blind to whether a scan they were manually segmenting was done before or after ECP. In order to control for variations in individual choroidal volume (the smoking group had larger mean choroidal volume than the other two groups, as can be seen below), changes were measured in percent rather than absolutes. It is unclear if varying delay between ECP-session and OCT measurements (Retinal Vessel Analysis and Flow Mediated Dilation were measured in between) could have distorted the results, considering findings of Sayin et. al., where choroidal thickness increased significantly 5 minutes after exercise, whereas measurements 15 minutes after exercise did not differ significantly from baseline.[38] In our study all post-ECP measurements, including the OCT, were performed directly after the ECP procedure and in the same order; that is, retinal vessel analysis, blood pressure measurement followed by the OCT. As such an abating effect on choroidal volume should have affected all groups roughly equally. Demarcation of the chorioscleral interface seemed to increase in difficulty on the more peripheral parts of the scan. We observed numerous irregular undulations of the chorioscleral junction, as has been reported previously.[11] Nonetheless, previous studies have shown excellent repeatability of manually segmented choroidal volume measurements in the outer ring of the abovementioned ETDRS grid.[11] Due to varying OCT scan quality (the chorioscleral junction had to be clearly visible), choroidal volume could not be calculated in several cases, making for a smaller sample size than initially intended. It may be for this reason that choroidal volume changes of the smoking subpopulation showed no statistical significance although subfoveal choroidal thickness (correlating with choroidal volume) significantly increased in the smoking group. Furthermore, choroidal volume increased significantly in diabetics, while subfoveal choroidal thickness change did not show statistical significance for the examined diabetics.

4.3 Conclusion

External counterpulsation may lead to an short term augmentation of choroidal volume in non-smoking, otherwise healthy individuals as well as in early diabetics. This effect seems to be less in smokers.

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